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A PHYSIOLOGICAL STUDY OF TWO STRAINS OF FUSARIUM IN THEIR CAUSAL RELATION TO TUBER ROT AND WILT OF POTATO

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 219[1]

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(WITH THIRTEEN FIGURES)

There is little doubt among phytopathologists that members of the genus *Fusarium* play an important rôle in producing diseased conditions in many plants, both wild and cultivated. According to WOLLENWEBER (**41**), *Fusarium* spp. produce wilt in members of the following families: Liliaceae, Bromeliaceae, Musaceae, Solanaceae, Convolvulaceae, Leguminosae, Malvaceae, Linaceae, Cucurbitaceae, Cruciferae, Compositae, Araliaceae, Caryophyllaceae, and Pedaliaceae.

History

The genus *Fusarium* was established by LINK (**20, 21**) in 1809, and *Fusarium* species were reported on rotted and ring-discolored tubers by MARTIUS in 1842, HARTIG in 1846, and SCHACHT in 1856. PIZZIGONI (**29**) and WEHMER (**38, 39**) demonstrated by experimental inoculation that *Fusarium* species can bring about tuber rot. They referred to the *Fusarium* in question as *F. solani*. Others, however, among them FRANK (**11, 12**) repeating their work, obtained negative results so far as *Fusarium* species were concerned; while DE BARY (**6**) and many others regarded the *Fusarium* spp. as nothing more than obligate saprophytes.

[1] In cooperation with the Nebraska Agricultural Experiment Station.

The credit of first demonstrating experimentally the relation of *Fusarium* spp. to certain plant wilts belongs to SMITH (**34**), who found a *Fusarium* responsible for watermelon wilt. SMITH and SWINGLE (**35**) reported a potato wilt and tuber rot which they considered due to a *Fusarium* which they called *F. oxysporum*. They considered this organism identical with *F. solani* of PIZZIGONI and WEHMER, and used the oldest name available, *F. oxysporum* (SCHLECHTENDAHL, 1824); however, they reported no experimental inoculations. PETHYBRIDGE and BOWERS (**28**) reported a dry rot due to *F. solani*, and LONGMAN (**22**) also reported a dry rot due to a *Fusarium*.

Many pathologists and mycologists entertained considerable doubt as to the parasitic nature of *Fusarium* spp., while others were quite convinced of their parasitic nature. SORAUER (**36**) was quite positive in his decision, while MASSEE (**25**) wavered. LINDAU (**18**) remained skeptical and referred to the *F. oxysporum* of SMITH and SWINGLE as a "Mischart." DUGGAR (**10**) was quite positive in his decision. Much of this difference of opinion undoubtedly was due to the confusion that prevailed as to the status of *F. solani*, *F. oxysporum*, and the genus in general, since no basis for extended morphological study of the genus had been established, and even the genus itself had not been sharply defined. MASSEE (**25**) considered *F. solani* to be the conidial form of *Nectria solani;* while REINKE and BERTHOLDT (**30**) considered it the conidial form of *Hypomyces solani*. LOUNSBURY (**23**) tried to arbitrate the matter by suggesting that *F. solani* and *F. oxysporum* are one and the conidial stage of *Nectria solani*. APPEL and WOLLENWEBER (**5**) published a monograph in which they defined the genus and brought some order into the chaos of species. Among other radical changes they dropped *F. oxysporum* and established *F. orthoceras* in its place.

MANNS (**24**) demonstrated by experimental inoculation that a *Fusarium*, which he designated (following SMITH and SWINGLE) as *F. oxysporum*, could produce tuber rot and wilt. He made no morphological studies, however, and undoubtedly had not had access to APPEL and WOLLENWEBER'S monograph.

JAMIESON and WOLLENWEBER (**16**) published an account of a dry rot of tubers induced by a *Fusarium* which they described as a

new species (*F. trichothecioides* Wr.). They refer to it as "a wound parasite capable of destroying potato tubers" and say "this disease is clearly differentiated from the wilt and dry rot ascribed by SMITH and SWINGLE to *F. oxysporum*."

Later, the writer (**19**) submitted his studies of a dry rot occurring among Nebraska potatoes as a thesis to the Graduate Faculty of the University of Nebraska. The work was done at the request of Dr. E. MEAD WILCOX, and consisted in part of a study of the morphology of a *Fusarium* that had been isolated from dry rotted tubers in 1908 by Miss VENUS W. POOL from potatoes that farmers had sent in from throughout the state during the season 1907–1908. Miss POOL established the causal relation of this *Fusarium* to the dry rot by experimental infection, and named the organism in manuscript *F. pulverulentum*, because of its powdery habit of growth. Both field and laboratory work were carried on for several years, and it was found that this organism caused primarily a dry rot of the tuber, and that it was not the *F. oxysporum* of SMITH and SWINGLE, a culture of *F. oxysporum* having been furnished the laboratory for comparative work through the courtesy of Dr. SMITH. The results were to have been published in 1911, and the organism was to be named *F. pulverulentum*, but upon the appearance of APPEL and WOLLENWEBER'S monograph Dr. WILCOX proposed to the writer that he reinvestigate the organism along the lines suggested by these authors. This was especially desirable since *F. oxysporum* had been dropped and several new species established. Not only was this carried out, but the whole etiology was gone over again and all of Miss POOL'S results verified. It was found that APPEL and WOLLENWEBER (**5**) had not described the species, and consequently it was described as *F. tuberivorum* Wilcox and Link (**40**). It was so named because of the apparent restriction of its activity to tubers.

A comparison of this paper and the paper of JAMIESON and WOLLENWEBER (**16**) made it seem quite likely that both were dealing with the same organism. The organism was isolated in the Washington laboratories from potatoes sent in from Washington, Nebraska, and other states in 1910, and WOLLENWEBER upon his arrival in the laboratory, using his monograph as the basis, described

it as a new species. He told the writer in 1913 that he felt convinced that we had described one and the same thing. Comparative studies made by the writer during the past year verify this point of view, and since the Nebraska publication by WILCOX, LINK, and POOL (**40**) did not appear in print until 1913, the name *F. trichothecioides* should be adopted.

WOLLENWEBER (**41, 42**) published a further paper in which he categorized the *Fusarium* spp. very sharply, dividing the genus into sections on the basis of physiological (that is, pathogenicity) and morphological (that is, conidia and chlamydospores) characters. *F. oxysporum* was again established and taken as the representative of the section ELEGANS, which comprises vascular parasites; and *F. trichothecioides* was put into the section DISCOLOR, which comprises parenchyma destroyers. He distinguished sharply between these and also between the vascular ring-discoloring *Fusarium* species of section ELEGANS and the tuber-rotting *Fusarium* species of sections DISCOLOR, GIBBOSUM, MARTIELLA, etc.

Referring to the papers by SMITH and SWINGLE (**35**), MANNS (**24**), and others, particularly to that by MANNS, he writes: "They do not separate fusarioses causing tuber rot from those causing both the wilt diseases of the plant and ring discoloration of the tuber, so that the reader might conclude that both wilt disease and tuber rot are caused by the same organism." Referring to his own experiments, he writes: "It also brings out the striking fact that the fungus, a typical xylem inhabitant, does not entirely destroy the tuber without the help of tuber rot *Fusarium* or bacteria," and "the fact that *F. oxysporum* causes the wilt of growing potato plants and only uses the xylem of the stem end of tubers for over-wintering, without producing a rot of the parenchyma, leads to interesting comparisons with the following 4 species which are able to destroy the tuber entirely from artificial wounds, namely, *F. coeruleum* (Lib.), *F. trichothecioides* Wr.," etc., and finally "the fact that the latter (*F. oxysporum*) cannot produce a tuber rot gives a biological contrast to the wound parasites of the tuber, and the fact that they cause the wilt disease of the growing plant presents a contrast to the saprophytes."

Problem and method of attack

In the spring and summer of 1914 the writer discussed the *Fusarium* situation as outlined by WOLLENWEBER with Dr. E. M. WILCOX and Dr. WILLIAM CROCKER. The former suggested that the whole situation ought to be gone over, and the latter that it would be of interest to search for the physiological basis of this alleged biological contrast. It is clear that, if the strict categories of WOLLENWEBER exist, then potato parenchyma must possess either an absolute or an effective immunity toward *Fusarium* spp. of the ELEGANS section, and that *Fusarium* spp. of the DISCOLOR section are either absolutely or practically unable to produce vascular mycoses or wilts.

The purpose of this research was twofold: (1) to determine whether such a sharp biological contrast exists; and (2) to determine what is the physiological basis for such a contrast. Experimental infections of potato plants and tubers were used for the first phase of the problem. It was clear that the second phase might involve a great many considerations, such as the structural, compositional, and metabolic nature of both host and parasite, as well as the relation of environmental factors to these. The important rôle played by the structural and compositional peculiarities of the potato and the influence of external factors upon these is well illustrated by the studies of APPEL and KREITZ (**1, 3**) on the efficacy of the cork layer in checking bacterial invasions of the tuber. Considerations of time and equipment limitations made it obligatory that the scope of the work be limited to a study of a few representative strains of the groups.

The writer is under obligation to the Departments of Agricultural Chemistry, Horticulture, and Experimental Agronomy of the University of Nebraska Experiment Station for the use of materials and equipment; to Miss ETHEL BEATY for help in much of the laborious routine; to Dr. FLORENCE A. MCCORMICK for valuable help in the anatomical and microtechnical phases of the problem; and to Mr. R. A. DAWSON for help in preparing the photographs.

The writer decided to work with *F. oxysporum* as representative of the vascular parasite section (ELEGANS), and with

F. trichothecioides or *F. tuberivorum* as representative of the parenchyma-invading section (DISCOLOR). Since it was desirable that the identity of the organisms be well established, the writer asked Mr. W. A. ORTON, in whose laboratories Dr. WOLLENWEBER had carried out his recent work, for cultures of the organisms. It was impossible to get cultures which had been authenticated by Dr. WOLLENWEBER, since he had gone to war, but through the courtesy of Mr. ORTON, MR. CARPENTER (Dr. WOLLENWEBER'S assistant) furnished a strain of *F. trichothecioides* (no. 41, 1916) and a strain of *F. oxysporum* (no. 3345A). The other strains of *F. trichothecioides* used had been isolated by the writer in 1911, and were described as *F. tuberivorum*. Several strains of *F. oxysporum* isolated from Nebraska potatoes were also used.

Pure cultures of these organisms were maintained on sterilized rice in plugged Erlenmeyer flasks, and these were used as a point of departure for all the work recorded.

I. Infection experiments

(1) EXPERIMENTAL INFECTION OF TUBERS

Tubers of the Early Ohio and Red Cobbler varieties were used in these experiments. Only sound tubers were selected, and these were thoroughly cleansed and sterilized before infection. At first they were sterilized by the formaldehyde gas method recommended by WOLLENWEBER (**41**). Several difficulties were encountered in using this method. It was found very difficult to remove the last traces of the gas without contaminating the chamber, and the tubers often showed the characteristic formaldehyde vapor injuries that have been discussed in bulletins of the New York Experiment Station (**13, 37**). Consequently, the writer abandoned the first method and sterilized tubers by immersing in 1:1000 $HgCl_2$ solution for 1.5 hours. Inoculation was carried out by removing a piece of the cortex with a sterile cork borer, placing an infected grain of rice into the hole, and then replacing the piece of tuber tissue. The wound was then sealed with sterile grafting wax and the tuber placed into sterile chambers. This proved an efficient and convenient way of carrying out the great number of experimental inoculations made.

The first inoculations were made in December 1914. The cut ends of 20 Early Ohio tubers were wetted with spore suspensions of *F. trichothecioides* and 5 tubers were kept as controls. Four inoculated and one control potato were kept in each compartment at a temperature of 25° C. in an almost saturated atmosphere. After 4 weeks all of the inoculated tubers were in advanced stages of rot.

On January 31, 3 potatoes were inoculated according to the second method with *F. oxysporum*, and 3 with *F. trichothecioides*, and kept at 20° C. until February 17. Two of the former set were slightly rotted and one totally, while the entire latter set was rotted severely. The controls showed no rot (fig. 1).

On January 15 another series was started which was kept at a temperature ranging from 15–20° C. until February 15. Six sets of 3 tubers each were started and each set was kept in a separate sterile chamber, 2 tubers of each set being inoculated by smearing cut surfaces with agar grown inoculum. Sets I, II, and III were inoculated with *F. oxysporum*, and sets IV, V, and VI with *F. trichothecioides*. In set I, one inoculated tuber was rotted, while the other and the control were sound; in set II, one was deeply rotted and the others sound; in set III, one was deeply rotted and the others sound; in set IV, two were rotted and the control sound; in set V, two tubers were rotted slightly and the control sound; and in set VI, two tubers were rotted and the control sound.

F. oxysporum and *F. trichothecioides* were re-isolated from these rotted tubers by placing tissue cut from such tubers on plated glucose agar. Nothing other than the organism with which the tuber had been inoculated developed. Inoculum from these plates was used in infecting tubers again with the same results.

Since these results were at variance with the statements of WOLLENWEBER the experiments were repeated with hundreds of tubers, and the results were verified.

DISCUSSION.—Tubers inoculated with *F. oxysporum* did not develop the ring discoloration that is considered characteristic of the activity of *F. oxysporum*, but a general rot of the whole tuber. Generally, however, this was not a dry rot, but a rot that resembles more the soft rots of bacterial origin, although it is not accompanied

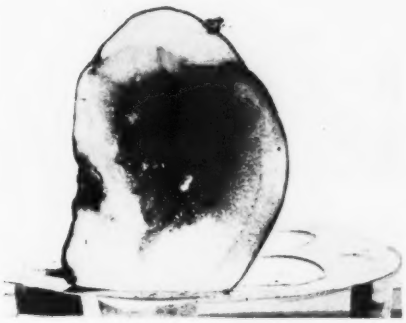
by the offensive odors of bacterial rots, producing a blackening and softening of tissue which extends a considerable distance beyond the actual site of the organism. This was demonstrated microscopically and culturally. At times, however, especially in cold, dry conditions, a dry rot as typical as that produced by *F. trichothecioides* was produced. *F. trichothecioides* invariably produced a dry rot with only a very limited darkened zone extending beyond the destroyed zone, made up of large cavities and a mixture of disintegrated, dry, shrivelled tissue and fungus tissue. No darkening extended beyond the actual site of the fungus and no softening of tissue occurred. Microscopic examination revealed the fact that *F. trichothecioides* attacked the tissue intracellularly and destroyed each cell completely before it proceeded to the neighboring cell, while *F. oxysporum* attacked the tissue intercellularly at first, and then attacked the cells intracellularly, but not until the tissue had been blackened and disorganized. In this way a softened tissue without cavities was produced. These rots produced experimentally with pure cultures of *F. oxysporum* lend support to the observations and conclusions of SMITH and SWINGLE (**35**), MANNS (**24**), who reports the occasional appearance of black specks in the parenchyma of tubers infected with *F. oxysporum*, and JONES (**17**), who attributes stem end rot of tubers to the activity of this organism, although they may have dealt with "Mischarten." SHERBAKOFF (**33**) reports certain strains of species of ELEGANS (using the section as a morphological group) to be tuber rotters. He distinguishes between *Fusarium* spp. that are tuber rotters and such as are vascular element inhabitants.[1]

(2) EXPERIMENTAL INFECTION OF LIVING PLANTS

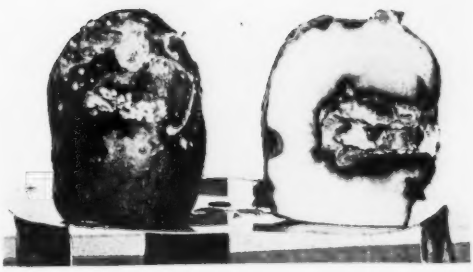
A series of experimental inoculations of healthy potato plants with *F. oxysporum* and *F. trichothecioides* were carried out, in an attempt to determine whether or not *F. trichothecioides* is unable to

[1] After these experiments had been concluded and this paper written, a paper by CARPENTER (**7**) has appeared. This represents a wholly independent although simultaneously conducted piece of work. The results of CARPENTER make it quite probable that the observations made by the writer on a few strains of *F. oxysporum* are of quite general application, since he arrives at the same conclusions for numerous though different strains of *F. oxysporum*. His conclusions as to the method of attack by the fungus and the nature of the rot are practically identical with the writer's.

produce wilt, or whether the potato plant enjoys an effective or practical immunity rather than an absolute one. Even though WOLLENWEBER (41) did not consider *F. trichothecioides* a wilt



A



B C

FIG. 1.—Tuber rot produced in laboratory with *Fusarium oxysporum*, and *F. trichothecioides*; *A*, soft rot produced by *F. oxysporum*, incubated at 20° C. for 17 days, Early Ohio variety; *B*, exterior of tuber rotted by *F. trichothecioides*, incubated at 20° C. for 17 days, Early Ohio variety; *C*, dry rot produced by *F. trichothecioides*, incubated at 20° C. for 17 days, Early Ohio variety.

producer in his 1913 paper, there is a reference in the 1912 paper by JAMIESON and WOLLENWEBER (**16**) to a wilt produced by *F. trichothecioides*. They referred to inoculation experiments, and report wilting in 12 days, "accompanied by a yellowing of the leaves and a discoloration of the tissue." The results of all of the writer's attempts of 1911–1912 to produce wilting of potato plants with *F. trichothecioides*, excepting one, were negative. During the past winter, however, it was noticed again and again that sprouts of tubers experimentally infected with this organism were dying. Microscopic and cultural studies left no doubt that this organism was responsible for the death of the sprouts.

Encouraged by these observations, the writer carried out some preliminary experiments on potato plants. Quartz was sterilized in 6 inch flower pots in the autoclave, and 8 plants that were about 10 cm. high were transplanted into these, the stems of some being smeared with rice infected with *F. trichothecioides*, and those of others with rice infected with *F. oxysporum*. The plants so inoculated and the controls were kept under bell jars. In three days the three plants smeared with *F. oxysporum* and two smeared with *F. trichothecioides* were dead, while the third one of the latter set and the controls remained healthy. The experiment was also conducted with potato plants growing in the open bench in the greenhouse, with similar results. The soil in this case was not sterilized.

The potato plants used in the following experiments were grown from sterilized tubers of the Early Ohio and Red Cobbler varieties in soil in 6 inch pots which had been thoroughly sterilized by heating in an autoclave for 4 hours on two consecutive days at 15 lb. pressure. The soil was watered with sterile water throughout the experiments.

On February 15, fifteen pots were planted with Early Ohio tubers and the soil of one set of 5 was infected with rice infected with *F. oxysporum*, of another with rice infected with *F. trichothecioides*, while the third set was left as a control. The controls came up in due time, while not a single one of the others came up. This experiment was repeated several times, but in no case was so striking a result obtained, although it often happened that some sprouts showed lesions, that some failed to come up, and that some were

tardy in coming up. *Fusarium* spp. were isolated from such lesions. These lesions are identical in appearance with lesions found on potato stems and roots in the field which often are designated as "foot disease" and ascribed to the activity of *Rhizoctonia*. Late in May other series were started and the soil was infected with rice and spore suspensions. No infections resulted at all, even though the inoculum was derived from the same source as that used in earlier experiments.

On March 12, sprouts that were just breaking through the ground were uncovered and smeared with rice infected either with *F. oxysporum* or with *F. trichothecioides*, 6 sprouts being used in each set. The plants were wounded no more than was inevitable in removing the soil. The soil was then replaced. The soil in the controls was removed in the same way, but no inoculum was applied. The 12 sprouts to which inoculum had been applied were killed, while the controls remained healthy. There was no spreading of the disease to other sprouts, even where an abundance of inoculum was applied.

The affected sprouts reminded one forcibly of affected sprouts in potato fields in the spring. Here and there in the fields one finds sprouts that look sickly and small, which usually wilt and die or remain sickly and small. Upon examination of such sprouts, prominent brownish, watery lesions are found. At times such sprouts overcome the trouble and make a fair growth, at least until transpiration becomes excessive. These lesions also account for many of the "poor stands" or failures of potatoes to come up evenly. If one digs in where a sprout ought to have come up, one can often find a tuber that has sprouted, but whose sprouts have been cut off entirely by such lesions. Often lateral buds develop into branches on such decapitated sprouts, only to be cut off again. If such a tuber finally manages to get a shoot above the ground, the shoot is sickly and backward. In 1912, 1913, and 1914 the writer plated the inner tissue of many such sprouts and almost invariably obtained cultures of various *Fusarium* spp., although often associated with *Rhizoctonia* and bacteria. Infection experiments conducted with *Rhizoctonia* in 1912–1913 gave almost uniformly negative results. The writer was at first inclined to refer

the major part of the potato troubles to the activity of this organism. Even though it is not the sole or even the main cause of Nebraska potato troubles it may play an important rôle. The work of APPEL (2), CORSAULT (8), DRAYTON (9), and MORSE and SCHAPOVALOV (26) gave results similar to those obtained by ROLFS (31, 32).

A *B*

FIG. 2.—Wilt produced in laboratory with *Fusarium trichothecioides*, and control plant; *A*, control, Early Ohio variety; *B*, wilting and drying of leaves, 4 days after inoculation, Early Ohio variety.

On March 13, 24 plants grown in sterile soil were used in another experiment. These plants were about 10 cm. high at the time. The soil was removed from one shoot in each pot and the pots were arranged in 6 series. In series *A* the shoots were wounded and the wound smeared with *F. oxysporum* infected rice; in series *B* the sound stem was smeared with *F. oxysporum* infected rice; in series *C* the wounded shoots were smeared with *F. trichothecioides* infected rice; in series *D* the sound stems were smeared with *F. trichothecioides* infected rice; in series *E* no inoculum was applied to the wounded shoots; in series *F* the soil was merely removed and replaced (figs. 2 and 3).

On March 15 the following notes were taken. Series *A:* plant 1, slight curling of leaves; 2, apparently sound; 3, curling of leaves; 4, curling of leaves. Series *B:* plant 1, drooping leaves; 2, lower leaves drooping, upper leaves drying; 3, apparently sound; 4, apparently sound. Series *C:* plant 1, apparently sound; 2, some wilting; 3, some wilting; 4, some wilting. Series *D:* plants 1, 2, and 3 apparently sound; 4, wilting. By March 21 the plants

FIG. 3.—Wilt produced in laboratory with *Fusarium oxysporum*, and control plant; *A*, control, Early Ohio variety; *B*, wilting of lower leaves and curling of upper leaves, 4 days after inoculation, Early Ohio variety.

infected with *F. oxysporum* showed a pronounced folding upward of leaves on the midrib, wilting and rolling on the margins of the leaves, the folding being most pronounced in the tips of the plants. The plants affected least showed discoloration on the margins, which at times was of a yellowish tint, at times purplish to violet. The leaves of plants most severely affected showed a yellowing and burning of the leaf margins. One plant, inoculated with *F. oxysporum*, developed a pronounced rosette, but overcame this later, growing into quite a normal plant (figs. 4 and 5). These symptoms remind one forcibly of certain symptoms of the leaf-roll disease which has received so much attention, and which has been made the subject

of thorough study by APPEL and his co-workers (**2, 4**). Eventually the plants infected with *F. trichothecioides* showed much severer symptoms than those inoculated with *F. oxysporum* (fig. 6). Eight plants died in the former sets, and 3 in the latter. Plants infected with *F. trichothecioides* showed such severe and rapid burning and

FIG. 4.—Leaf roll and rosette of field plant of the Pearl variety; August 1912, at the U.S. Substation at Mitchell, Neb.

drying up of leaves, that the typical wilting phenomena were scarcely realized. The vascular bundles were blackened and the blackening extended even into the petiole and the leaf veins. This rapid killing was at first strictly localized on that side of the plant to which the inoculum had been applied, even in the leaf, where the leaflets on one side of the midrib would be affected, and those on the other side not. Eventually in those cases in which killing of the

whole plant took place, the fungus girdled the whole stem, while plants that were not girdled lived on, even though one side was entirely destroyed. There was little lateral and subsequent vertical spreading of the fungus from one vascular strand to the other. These experiments were repeated with 25 other plants and in most cases the same symptoms were observed. These symptoms have been repeatedly observed in the dry land areas of Nebraska,

A *B*

FIG. 5.—Rosette produced in laboratory with *Fusarium oxysporum*, and control plant; *A*, control, Early Ohio variety; *B*, rosetted plant, 10 days after inoculation, Early Ohio variety.

but have always been looked upon as cases of "sun scald," and and in previous experiments with wilting due to *F. trichothecioides* such cases were ignored.

Plants grown in soil infected with *F. oxysporum* and *F. trichothecioides* showed severe lesions of root and stolons. Examination of roots affected with either organism showed that the cortical regions are first and most severely attacked, not only intercellularly, but also intracellularly, the cells being packed full with hyphae. In most cases the cortex could be sloughed off with exceeding ease. From the cortex the organisms invaded the stelar regions, where

F. oxysporum makes greater headway than the other and there causes a vascular mycosis more frequently, which accounts for its designation as a vascular parasite (figs. 7 and 8).

DISCUSSION.—If plants, experimentally inoculated, showed only light symptoms to begin with, most of them continued their growth with symptoms less severe than those shown in the field. If they showed severe symptoms early, these proved more severe and

A *B*

FIG. 6.—Wilt and death of potato plants produced in laboratory with *Fusarium trichothecioides*, 12 days after inoculation; Early Ohio variety; wilting is restricted to the side to which inoculum was applied.

more rapidly fatal than those in the field. The organisms in the field work much more insidiously, attacking the roots of the plant slowly but progressively, and permitting the plant, except in extreme cases, to readjust for its water requirements. These readjustments manifest themselves in the curling and rolling phenomena (figs. 4 and 9).

Potato plants in the irrigated sections show this phenomenon nicely. As long as cultivation and irrigation are maintained, the plant develops new roots progressively higher up, and the infected

plants get along fairly well, showing slight curling and wilting, although tuber development occurs. When in midsummer

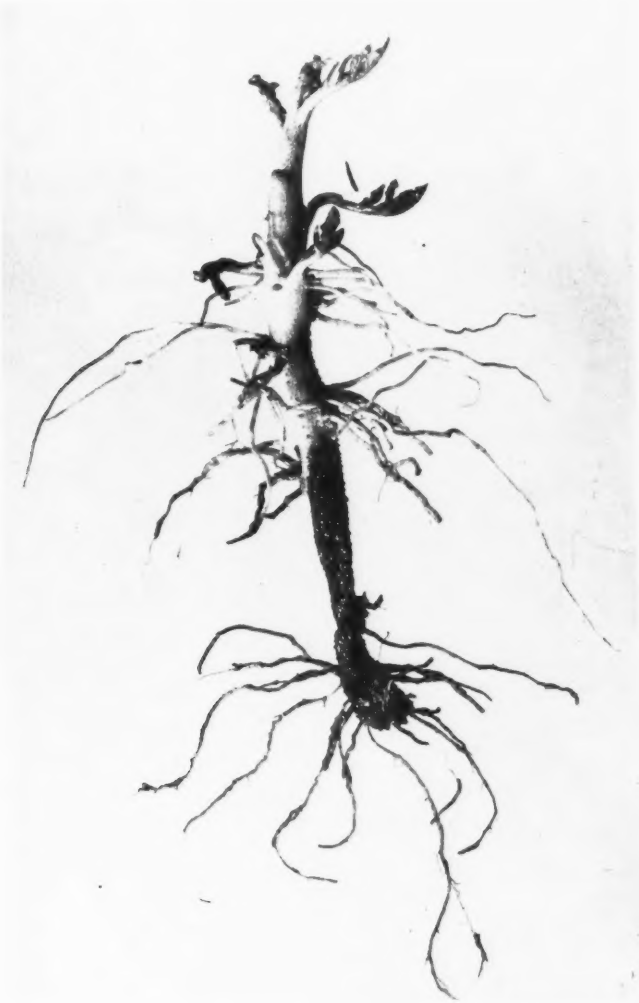


FIG. 7.—Lesions on stem and roots produced in laboratory with *Fusarium oxysporum*, 2 weeks after inoculation; Early Ohio variety.

FIG. 8.—Root lesions produced in laboratory with *Fusarium trichothecioides*, curling and rolling of leaves, 2 weeks after inoculation; Early Ohio variety.

irrigation ceases and no more soil is heaped about the crown of the plant and transpiration requirements must be met by badly infected

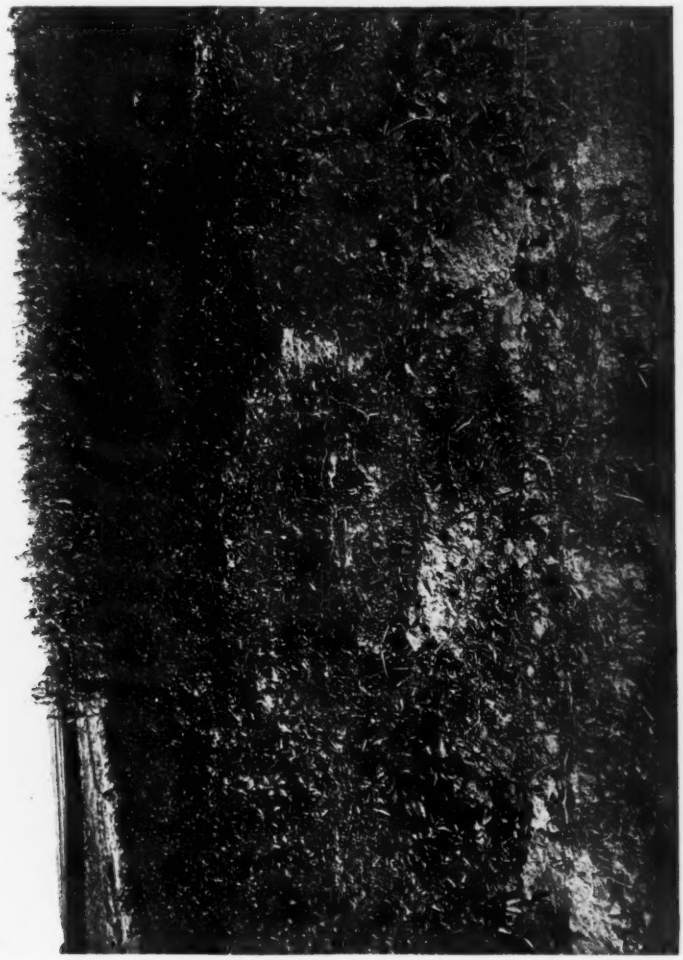


FIG. 9.—Potato wilt and little potatoes in a field of the Pearl variety, August 1912, at Mitchell, Neb.

lower roots and a few healthy upper ones without the possibility of developing new roots, the plant soon succumbs. In this way we get the exceedingly frequent phenomenon of large plants, usually

with many small tubers, wilting down suddenly after the last irrigation. The frequent occurrence of aerial tubers, the prevalence of excessive numbers of small tubers, and the occurrence of few abnormally large tubers on such plants is also attributable to the insidious manner of attack. The organisms attack the stolons and main stem as well as the roots. Stolons with tubers in all stages of maturity can be found partially or completely cut off by lesions. As the balance between the photosynthetic and storage centers in such plants is disturbed, new stolons are developed nearer and nearer the surface and the stolons that are not attacked develop abnormally large tubers. Often the plant responds to this disturbance in the assimilation-storage balance by producing swellings of the aerial parts of the plant, the so-called aerial tubers. Many large plants can be pulled up with ease, because lesions make separation of the tops from the roots or even the basal portion of the stem easy. Such plants may show a comparatively sound main axis (fig. 10).

Infection carried over by the mother tuber, which is frequent, rarely permits the growth of stems more than 20 cm. high, and seldom allows the development of tubers. An early attack from without upon the main stem leads to equally disastrous results.

The wilts of the potato plant induced by *Fusarium* spp. have generally been considered vascular mycoses due to a clogging of the vascular elements. In fact, however, the symptoms are due to killing of the root system as much as to clogging of the vascular elements. It is true that members of the ELEGANS section, such as *F. oxysporum*, frequent the vascular elements, spreading in these rather than clogging them, but it is true also that they destroy roots in numbers. Again, even though some have referred to this disease as a root disease (SMITH and SWINGLE **35**), it is stated that the fungus enters a root, then spreads to the stelar part, and from there enters other roots and stolons. Just as much damage is done by the persistent attack from without upon roots and stolons, as noted by MANNS (**24**).

In the course of these experiments several questions were raised. The soil in these experiments surely was more severely infected with the organisms than soil under field conditions can

FIG. 10.—A plant of the field shown in fig. 9; after showing slight curling and discoloration of leaves and formation of aerial tubers in earlier part of summer this plant suddenly wilted down and died; such a plant as this usually bears a great number of small and a few abnormally large tubers which are infected in the stem end with the fungus that killed the mother plant.

be, yet there were many plants grown in such soil that showed no infection whatsoever. Less success in producing wilt was observed as the season progressed. It remains a question whether this is due to a loss in vitality or to a gain in resistance in the plants, due to a change in the soil, tubers, or the organism.

Whether the success in producing wilt with *F. trichothecioides* and the apparent waning of this power is due to a gain or regain of virulency and a subsequent loss again is also an unanswered question. SHERBAKOFF (33), working with *Fusarium* spp., got uniformly negative results so far as producing wilt is concerned, and concluded that the results were due to a loss of virulency of the cultures or to some other important factor that had escaped attention.

Summary

It is quite apparent that some of the strains of *F. oxysporum* can cause tuber rot; that they can destroy tubers entirely without the aid of other *Fusarium* spp. or bacteria; that at least one *Fusarium* of the DISCOLOR section (*F. trichothecioides*) can produce wilt of stem; and that the biological contrast drawn by WOLLENWEBER between the *Fusarium* spp. is not as sharp as one would infer from his paper. It is possible that these strains of *Fusarium* spp. are morphologically identical with those described by WOLLENWEBER, but physiologically unlike them. That this rule, if it exists, is not so rigid generally, however, is noted by SHERBAKOFF (33), who found that no correlation exists between morphological relationship and pathogenicity.

Although *F. oxysporum* is not absolutely unable to attack potato parenchyma, the potato tuber, in which usually only the xylem elements are invaded, enjoys an effective immunity from its attacks; and although *F. trichothecioides* can attack any subterranean part of the living potato plant, generally all parts excepting the mature tuber enjoy an effective immunity from its attacks.

The data given in the second part of this paper may furnish a partial explanation of these phenomena.

II. Ecology and physiology of the organisms

METHOD AND DATA

1. TEMPERATURE RELATIONS.—Observations of cultures grown at ordinary temperatures showed that there is a striking difference in the rate of growth of the two organisms. Potato cylinder, rice, liquid potato, and glucose media, and glucose and potato agar cultures all showed that *F. oxysporum* makes a considerably greater initial growth at temperatures above 20° C. than does *F. trichothecioides*. At temperatures in the vicinity of 10–15° C., however, *F. trichothecioides* makes the greater initial growth, although these temperatures lie below its optimum. The same difference was noted in cultures on neutral and acid potato agar. This point was also tested with cultures on sterile slabs of potato tubers kept in Petri dishes. At 25° C. *F. oxysporum* covered such slabs completely when *F. trichothecioides* barely had made a start, while at 12° C. the situation was reversed.

When 1 per cent liquid glucose media were inoculated with spore suspensions of *F. oxysporum*, visible growth was made in 16 hours; when *F. trichothecioides* was used, 30–42 hours elapsed before visible growth was made. This holds for temperatures above 20° C. The optimum temperature for *F. oxysporum* was about 30° C., and for *F. trichothecioides* about 20–22° C., both varying slightly with the medium used. The maximum for *F. oxysporum* lay between 38 and 40° C. The optima and maxima were higher for cultures in potato extract than for glucose media cultures. The writer has not been able to determine the minima accurately because of inadequate apparatus. HUMPHREY (**15**) gives 4° C. as the minimum growth temperature for a certain strain of *F. oxysporum*.

Potato agar cultures of *F. oxysporum* and *F. trichothecioides* could endure a temperature of 40° C. for 5 and for 20 hours respectively and remain viable. Exposure at 50° C. for 5 hours killed *F. trichothecioides*, but not *F. oxysporum;* while exposure for 20 hours killed both. Some *F. oxysporum* cultures survived 5 hours exposure at 57° C.

The growth relations were also checked up quantitatively. In these experiments, as well as in all the following ones, the method

suggested by HASSELBRING (**14**) was followed. Erlenmeyer flasks of 200 cc. capacity were used with 50 cc. of solution per flask. The solutions in the flasks were autoclaved for 10 minutes at 7 lb. pressure, and then inoculated by means of sterile pipettes with a drop or two of spore suspension. The cultures were killed by adding 10 cc. of 10 per cent HCl to each flask. The cultures were then filtered off on tared Gooch crucibles prepared with asbestos, washed until acid free, and brought to constant weight in a Freas electric oven at 100° C., and the dry weight determined. It was found impossible at times to filter luxuriant cultures of *F. oxysporum* by this method, because of the tenacity with which this organism holds water. Consequently they were filtered on soft filter paper, transferred to tared Gooch crucibles, dried, and weighed. The other organism holds water with little tenacity and filters with ease.

In all of experiments given below the following stock mineral solution was used: 20 gm. NH_4NO_3; 10 gm. KH_2PO_4; 5 gm. $MgSO_4$ per 1000 cc. H_2O. When carbohydrates were employed,

TABLE I

DRY WEIGHT (IN MILLIGRAMS) AFTER 20 DAYS' GROWTH IN POTATO EXTRACT MEDIUM; ROOM TEMPERATURE

	FUSARIUM OXYSPORUM					
	Temperature					
	35°	30°	25°	12°	1°.11*	−1°.11*
Flask 1	40	55	63	64	62	86
Flask 2	47	78	68	66	.....	.....
Flask 3	61	86	80	68	.....	.....
Average	49	73	70	66	62	86

	FUSARIUM TRICHOTHECIOIDES					
	Temperature					
	35°	30°	25°	12°	1°.11*	−1°.11*
Flask 1	0	0	60	87	146	83
Flask 2	0	0	64	100	.....	.....
Flask 3	0	0	65	147	.....	.....
Average	0	0	63	111	146	83

*For 20 days (no growth), then at 25° C. for 25 days.

these were added at the rate of 10 gm. per liter. Potato extract medium was made up by extracting 500 gm. ground potato tuber with 500 cc. H_2O, and then adding 500 cc. of the foregoing solution to the extract.

A series of cultures (table II) was run at 12° C. and the amount of dry weight formed determined at 2 day intervals for 10 days. In this series the medium was at 12° C. at the time of inoculation.

TABLE II

DRY WEIGHT (IN MILLIGRAMS) IN POTATO EXTRACT MEDIUM; TEMPERATURE 12° C.

	FUSARIUM OXYSPORUM					FUSARIUM TRICHOTHECIOIDES				
	Number of days					Number of days				
	2	4	6	8	10	2	4	6	8	10
I.....	0.2	0.4	5.2	.....	13.2	1.4	4.0	9.4	.....	27.0
II.....	0.6	0.4	5.6	.....	13.0	1.8	4.4	12.2	.....	44.6
Average.	0.4	0.4	5.4	.....	13.1	1.6	4.2	10.8	.....	35.8

Table III shows the growth by day intervals made for 10 days when levulose was used as the carbon source. The solutions were at the temperatures indicated at the time of inoculation.

TABLE III

DRY WEIGHT (IN MILLIGRAMS) FORMED BY DAY INTERVALS

	FUSARIUM TRICHOTHECIOIDES AT 25°C.									
	Number of days									
	1	2	3	4	5	6	7	8	9	10
I.....	0.2	0.6	4.2	12.8	29.0	19.2	25.4	19.4	34.0	43.6
II.....	0.2	2.4	18.0	31.6	32.4	34.2	27.2	41.9	47.7	50.8
Average.	0.2	1.5	11.1	22.2	30.7	26.7	26.3	30.6	40.8	47.2

	FUSARIUM OXYSPORUM AT 25°C.									
	Number of days									
	1	2	3	4	5	6	7	8	9	10
I.....	0.4	1.8	9.2	16.0	23.6	19.6	24.0	24.1	30.9	37.0
II.....	0.8	3.2	10.8	29.2	33.4	21.2	27.5	26.0	34.8	38.8
Average.	0.6	2.5	10.0	22.6	28.5	20.4	25.7	25.0	32.8	37.9

TABLE III—*Continued*

	Fusarium oxysporum at 30°C.									
	Number of days									
	1	2	3	4	5	6	7	8	9	10
I.....	1.0	3.6	13.4	17.8	37.6	45.8	55.8	66.0	78.2	62.2
II.....	8.9	5.2	17.6	19.0	40.2	77.8	62.6	69.6	78.6	66.6
Average.	4.9	4.4	16.5	18.4	38.9	61.8	59.2	67.8	78.4	64.4

	Fusarium trichothecioides at 25°C.									
	Number of days									
	1	2	3	4	5	6	7	8	9	10
Average...	4.4	19	67.4	.....	.....	.....	.....	262	.....	.....

	Fusarium oxysporum at 30°C.									
	Number of days									
	1	2	3	4	5	6	7	8	9	10
Average...	11.2	48	108.6	.....	.....	.....	.....	240.2	.....	.....

These tables show a tendency of *F. trichothecioides* to make a greater initial growth at low temperatures. At higher temperatures, however, unless above the optimum of *F. trichothecioides*, *F. oxysporum*, even though it made the greater initial growth, was soon overtaken and passed by *F. trichothecioides*. This was especially marked when dextrose and levulose were used as carbon source. This may be the result of a faster though more superficial feeding of *F. oxysporum*, which makes it unable to use materials as thoroughly as the other organism. This phenomenon is hardly a case of more rapid intoxication on the part of *F. oxysporum*.

The results obtained with artificial media were verified by infection experiments conducted with potato tubers kept at various temperatures. Tubers of the Red Cobbler variety were used. These were inoculated on April 1, and examined on May 27 (table IV). See figs. 11 and 12.

It should be noted here that *F. trichothecioides* when inoculated into a tuber can grow at 30° C., while it cannot do so in artificial media; and that *F. oxysporum* can survive a temperature of 1° C. in artificial media, but not in the tuber.

TABLE IV

CONDITION OF TUBERS AT CLOSE OF EXPERIMENT

Temperature	Fusarium oxysporum	Fusarium trichothecioides
30° C.	All completely rotted; sprouts killed	Slight rot in some
25° C.	All completely rotted; sprouts killed	All completely rotted; some sprouts killed
12° C.	All with very slight rot	All completely rotted
1° C.	No rot	All with slight rot
1° C. for two weeks, then 25° C. for two weeks.	Slight rot in one tuber	All completely rotted
−1° C.	No rot	No rot
−1° C. for two weeks, then 25° C. for two weeks.	No rot	All completely rotted

DISCUSSION.—These results may, in part at least, explain why *F. oxysporum*, even though it can attack parenchyma and rot tubers, usually is not found in rotted tubers, while *F. trichothecioides* is. The ability of the latter to make a faster initial growth at the temperatures which prevail in the soil about digging time and in well kept storage places is probably the determining factor in this phenomenon. The experiments with tubers showed that *F. trichothecioides* made a great increase in growth rate when transferred from a low to a higher temperature.

These temperature relations may also explain in part the fact that we usually find *F. oxysporum* producing wilt under field conditions, and lend support to the observations made by ORTON (**27**), who reports potato wilt induced by *Fusarium* spp. to be preeminently a warm climate disease. *F. trichothecioides* can produce wilt, but the temperature conditions in the soil are such as to favor *F. oxysporum*, the maximum temperature of the former being the optimum of the latter. HUMPHREY (**15**), working in Washington on the tomato wilt induced by *F. oxysporum*, came to the conclusion that temperature differences in various parts of the state were determining factors for the appearance and non-appearance and severity of the disease.

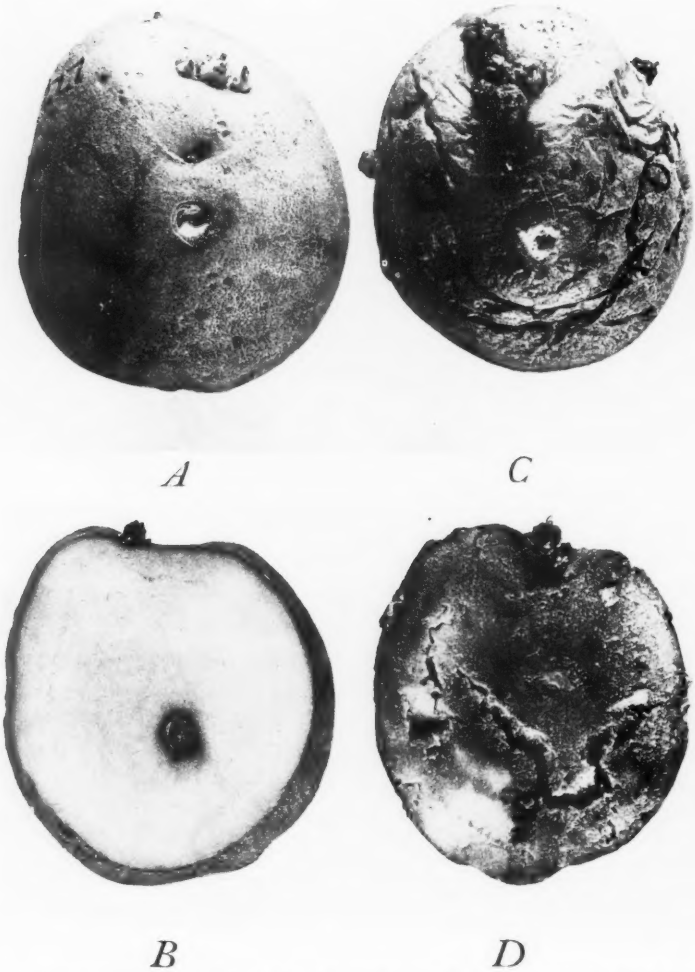


FIG. 11.—Tuber rot of Red Cobbler variety produced by inoculation with *Fusarium oxysporum*; *A*, *B*, external and sectional view of same tuber, incubated for 30 days 12° C.; *C*, *D*, external and sectional view of same tuber, incubated for 30 days at 25° C.

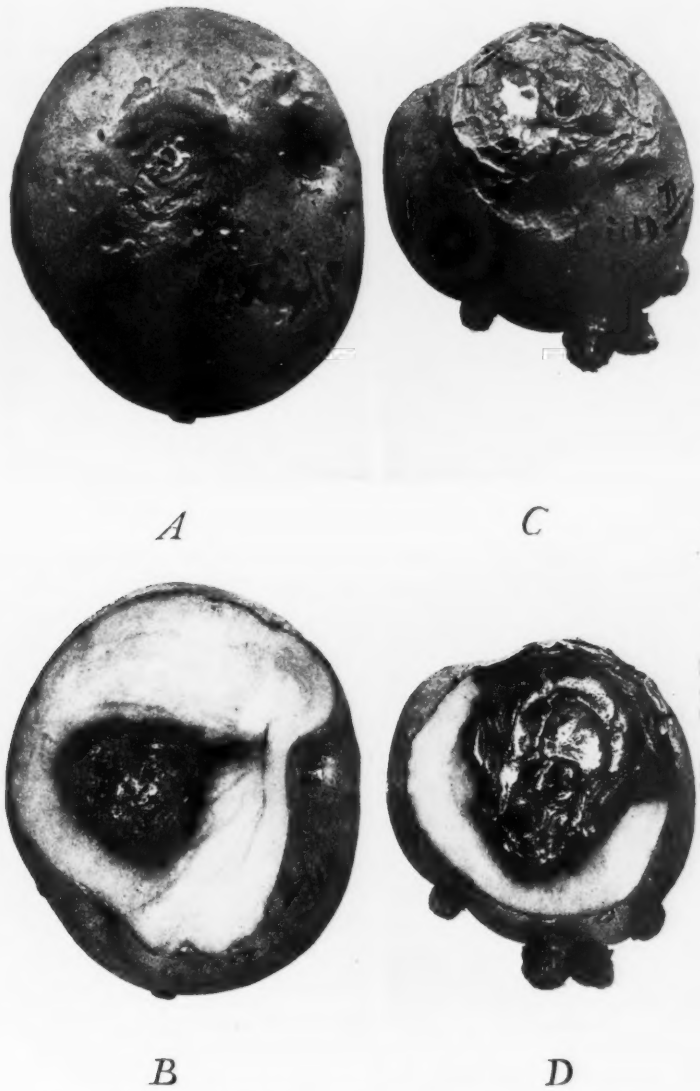


FIG. 12.—Tuber rot of Red Cobbler variety produced by inoculation with *Fusarium trichothecioides; A, B*, external and sectional view of same tuber, incubated for 30 days at 25° C.; *C, D*, external and sectional view of same tuber, incubated for 30 days at 12° C.

2. GROWTH HABIT.—It was observed in nearly all cultures that *F. oxysporum* not only made a greater initial growth at ordinary temperatures, but that it was at all temperatures much more of a surface grower than *F. trichothecioides*, making a superficial spreading growth, rather than the penetrating restricted intensive growth of the latter. Early sporulation was associated with the restricted growth habit of the latter (fig. 13). These habits were especially clearly marked on solid substrata, but even in liquid media *F. oxysporum* made a much less compact growth than the other species. It may be that the more spreading and extensive growth habit of *F. oxysporum* at all temperatures and its more rapid initial growth at temperatures above 10–15° C. are associated with a greater oxygen requirement than that possessed by *F. trichothecioides*. This would explain in part the frequenting of intercellular spaces and xylem elements by the former, and its consequent greater efficiency in causing vascular mycosis and wilt, as well as its tendency to cause bundle discoloration. The xylem elements of the stem end are undoubtedly infected while the tuber is yet in the soil, where temperature conditions are such as to favor the growth of *F. oxysporum*. Storage temperatures check the growth of this organism and the cells bordering the infected vascular elements shut the infected area off by suberizing their walls. Cultural experiments and microscopical studies show that cork is not absolutely impenetrable to these organisms, although it provides under normal conditions an effective barrier to the progress of both of these species. Because of the slower growth of *F. trichothecioides* at higher temperatures, the potato plant undoubtedly has a much better opportunity to guard itself by cork formation against this organism than against the other.

3. THE CARBON SOURCES OF THE TWO ORGANISMS.—A difference in the metabolic requirements of two organisms, a difference in their ability to utilize various substances, or a difference in their ability to tolerate the presence of substances may be factors of critical importance in determining which of the two will attack a given tissue or a given plant. These factors may determine also the modes of attack of an organism upon a tissue or a plant. Thus an organism that can digest pectinaceous material and not cellulose

would have to destroy a tissue whose walls are mainly cellulose by intercellular activity, while one that could digest cellulose might

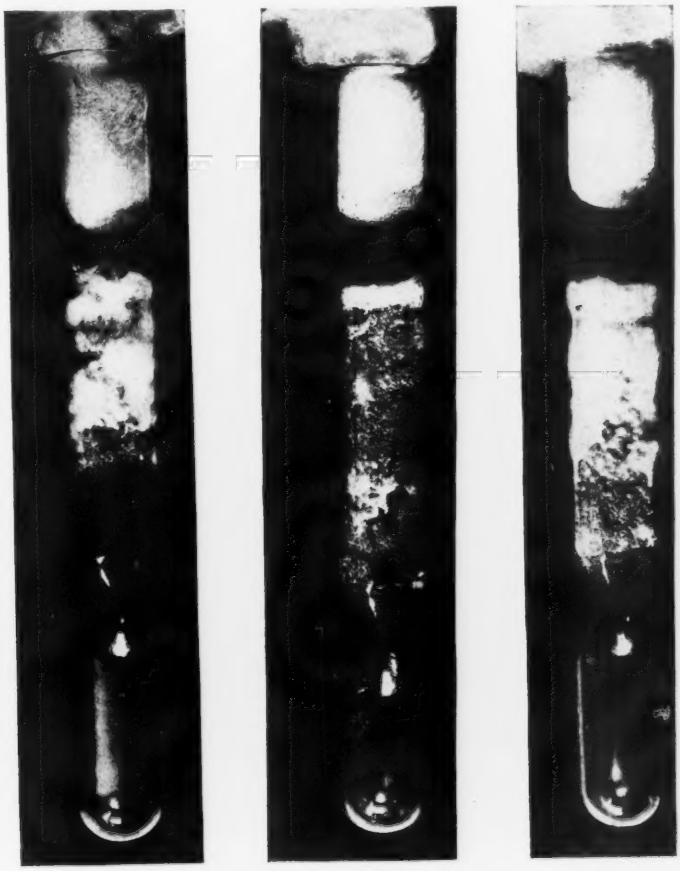


FIG. 13.—*Fusarium trichothecioides* and *F. oxysporum* on sterile potato cylinders; *A*, *C*, cylinders inoculated with *F. oxysporum*, incubated for 2 days at 25° C.; *B*, cylinder inoculated with *F. trichothecioides*, incubated for 2 days at 25° C.

destroy this tissue by a primary cell invasion. Again, a greater ability on the part of an organism to digest suberin, other things

being equal, would render it a much more formidable enemy of the potato plant than an organism without this ability, or possessing it to a less degree. This problem was attacked by making a study of the carbon sources of the organisms. The data reported here are only a beginning of this phase of the problem.

Fifty cc. of nutrient solution were measured quantitatively into 200 cc. Erlenmeyer flasks with a pipette. The flasks were then plugged with cotton, covered with tinfoil, and autoclaved. After cooling, 0.5 gm. of carbohydrate material was transferred quantitatively into each flask, and the flasks covered again with tinfoil and sterilized in a Freas oven by heating at 85° C. for one hour every 12 hours, for 6 consecutive days. The solutions were then incubated at 25° C. for 48 hours, so as to allow any contaminations present to appear. Low sterilization temperature was used to reduce hydrolysis of carbohydrates to a minimum.

The dry weight determinations were made by the methods outlined above. It was found advisable to kill two cultures of each set after 6 days, for the striking differences in rate of growth between the two organisms that were observed during the first 48–120 hours were obliterated by prolonged growth. The other 3 cultures were killed after 12 days' growth. The dry weight values do not show the differences in habit and rate of growth in the cultures as strikingly as they appeared to the eye. In many cases a visible growth was not determinable as dry weight. This is readily appreciated when we consider that moisture determinations indicated that the dry weight varied between 10 and 20 per cent of the wet weight.

In the controls, consisting of the plain mineral medium without carbon material, *F. oxysporum* made a weighable growth in 12 days, though not in 6 days, while *F. trichothecioides* made no weighable growth even after 12 days. Another important observation was made. In no case was it necessary to reinoculate with *F. oxysporum*, while many *F. trichothecioides* inoculations failed. The latter undoubtedly is the slower starter and much more poorly equipped for sure and quick infection than the former.

The figures in tables V–VIII represent milligrams of dry weight of material formed, except in those cases in which per cent is written. In such cases (cork, cellulose, and hemicellulose), the figures repre-

sent the percentage of decrease in dry weight of material. The last weighing in these cases unavoidably included the dry weight of fungus material formed, so that the figures are higher than they ought to be. The differences in weight in these cases give only comparative values of the amounts of material respired by the organisms. The filter paper used was the best Swedish paper, and the cork was obtained by skinning steamed potatoes, scrubbing the skin thoroughly, boiling it for 48 hours in distilled water, extracting for 48 hours in ether, and then boiling again with water. All figures represent averages, the composition of these figures being shown in tables VI and VII. In many cases there was a fair coincidence of the values, while in others a great disparity appeared. The averages probably would more nearly approximate the true value if a greater number of figures were available.

TABLE V

DRY WEIGHT (IN MILLIGRAMS) FORMED IN 6 AND 12 DAYS BY *Fusarium trichothecioides* AND *F. oxysporum*

	FUSARIUM TRICHOTHECIOIDES		FUSARIUM OXYSPORUM	
	Number of days		Number of days	
	6	12	6	12
Ethyl alcohol	.....	1.2	15.0	18.5
Glycerine	12.0	20.5	17.0	92.0
Mannit	108.5	136.3	109.5	112.0
Arabinose	5.2	35.0	78.7	43.0
Glucose	11.5	42.3	43.5	44.0
Mannose	2.0	52.0	49.0	62.0
Galactose	28.8	43.3	39.0	73.0
Fructose	56.5	81.0	74.5	77.6
Saccharose	9.5	45.6	36.5	35.6
Maltose	13.0	61.0	35.5	50.3
Lactose	1.3	20.0	4.0	21.0
Raffinose	12.4	47.3	44.5	54.6
Potato starch	.....	45.0 per cent	81.0	103.0
Wheat starch	22.6 per cent	42.1 per cent	54.3 per cent	127.0
Corn starch	19.7 per cent	33.4 per cent	37.9 per cent	67.2 per cent
Soluble starch	45.2 per cent	49.0 per cent	110.5	210.3
Dextrine	4.4	37.3	42.0	56.3
Inulin	63.6	123.0	102.0	90.0
Gum arabic	1.5	18.6	37.0	58.3
Gum tragacanth	67.5 per cent	14.1 per cent	27.1 per cent	25.7
Hemicellulose	10.1 per cent	7.3 per cent	10.1 per cent	6.7 per cent
Cellulose	4.0 per cent	1.0 per cent	4.25 per cent	6.7 per cent
Cork	10.1 per cent	5.9 per cent	12.8 per cent	3.9 per cent
No carbon source	.....	.....	.....	0.5

Table V shows that qualitatively the two organisms behave alike in their ability to use all the carbon compounds tested. Quantitatively there is considerable difference, both as to rate of consumption and total growth after 12 days. *F. oxysporum* in general shows the greater speed of growth and greater growth after twelve days. In some cases *F. trichothecioides* shows the greater growth after 12 days.

TABLE VI

DRY WEIGHT (IN MILLIGRAMS) FORMED WHEN EACH FLASK RECEIVED 0.5 GM. OF MANNIT; CONCENTRATION 1 PER CENT

NUMBER OF FLASK	FUSARIUM OXYSPORUM		FUSARIUM TRICHOTHECIOIDES	
	6 days	12 days	6 days	12 days
I	93	.....	107	.....
II	120	.....	110	.....
III	.....	108	.....	130
IV	.....	112	.....	135
V	.....	116	.....	141
Average	109.5	112	108.5	136.3

TABLE VII

HEMICELLULOSE (IN GM.) USED IN 12 DAYS

NO. OF FLASK	FUSARIUM OXYSPORUM			FUSARIUM TRICHOTHECIOIDES		
	6 days			6 days		
	Grams of material	Decrease in weight	Percentage of decrease	Grams of material	Decrease in weight	Percentage of decrease
I	0.5055	0.0455	9.0	0.5213	0.0513	9.8
II	0.5154	0.0584	11.3	0.4964	0.0524	10.5
Average	.....	.....	10.1	.....	.....	10.1
	12 days			12 days		
III	0.5185	0.0355	6.1	0.501	0.028	5.5
IV	0.5192	0.0382	7.3	0.5058	0.0488	9.4
Average	.....	.....	6.7	.....	.....	7.3

Table VIII gives the dry weight formed by the organisms when carbon acids were furnished as carbon sources. N/100 solutions were used, excepting for asparagin and asparagenic acid, whose solu-

bility permitted only N/200 solutions. In the case of the higher fatty acids and oils, the material was weighed out as though N/100 solutions were being prepared. With these no weighings of the material formed were attempted, but merely differences in luxuriance of growth were recorded. To those acids which showed no growth with N/100 solutions, 5 cc. of 10 per cent glucose solution was added, making the sugar concentration 1 per cent, so as to determine whether the acid was merely non-usable, or whether it was toxic. Since it was found that some were toxic at N/100 concentration, lower concentrations were made up also. The results are given in table IX.

TABLE VIII

DRY WEIGHT (IN MILLIGRAMS) FORMED WITH THE FOLLOWING CARBON COMPOUNDS AS CARBON SOURCES

	FUSARIUM TRICHOTHECIOIDES		FUSARIUM OXYSPORUM	
	Number of days		Number of days	
	6	12	6	12
Formic acid N/100....	None	None	None	None
+5 cc. 10 per cent glucose solution.....	"	"	"	85.5
Acetic acid N/100.....	"	"	1.2	10.6
+5 cc. 10 per cent glucose solution.....	"	106	None	None
Proprionic acid N/100..	"	None	"	"
+5 cc. 10 per cent glucose solution.....	"	"	"	"
Butyric acid N/100....	"	"	"	"
+5 cc. 10 per cent glucose solution.....	"	"	"	"
Glycocollic acid N/100.	2.5	7.3	20.0	8.6
Lactic acid N/100.....	0.3	0.6	8.0	23.0
Oxalic acid N/100.....	Non-weighable	Non-weighable	Non-weighable	Non-weighable
Succinic acid N/100...	Non-weighable	Non-weighable	8.5	9.3
Malic acid N/100.....	Non-weighable	Non-weighable	4.5	8.0
Tartaric acid N/100...	1.0	0.93	6.0	4.3
Citric acid N/100.....	2.6	6.6	8.0	6.6
Aspartic acid N/200...	1.6	.....	5.5	6.5
Asparagin N/200.....	3.5	3.8	5.5	5.3
Tannic acid 1 per cent.	1.0	31.0	0.2	42.0
Tannic acid 0.5 per cent	1.6	61.0	1.6	41.0
+5 cc. 10 per cent glucose.....	3.5	32.0	5.0	47.0
Control 0.5 gm. levulose.....	.....	82.0	.....	116.0

TABLE VIII—*Continued*

	FUSARIUM TRICHOTHECIOIDES	FUSARIUM OXYSPORUM
	12 days	12 days
Palmitic acid I	No growth	Good growth
" " II	Slight "	" "
" " III	" "	" "
" " IV	" "	" "
+5 cc. 10 per cent dextrose, V	Excellent "	" "
Stearic acid, I	Slight "	" "
" " II	No "	" "
" " II	No "	" "
" " III	" "	" "
" " IV	" "	" "
+ 5 cc. 10 per cent dextrose, V	Excellent "	" "
Oleic acid, I	No "	" "
" " II	Poor "	" "
" " III	" "	" "
" " IV	" "	" "
+5 cc. 10 per cent dextrose, V	Slight "	" "
Palm oil, I	No "	No "
" " II	" "	" "
" " III	" "	" "
" " IV	" "	" "
+5 cc. 10 per cent dextrose, V	" "	" "
Olive oil, I	Fair "	Fair "
" " II	" "	" "
" " III	" "	" "
" " IV	" "	" "

A marked difference was found in the ability of the two organisms to use the fatty acids, *F. trichothecioides* being much more restricted in its ability. The experiments with alcohol and the acids also showed that the former organism was much more readily poisoned and inhibited in its growth. It was found that *F. oxysporum* grew well in 1 per cent ethyl alcohol, and that *F. trichothecioides* made no growth. The solution was then diluted one-half, whereupon *F. trichothecioides* made a good growth. This was clearly a case of inhibition. The growth of *F. trichothecioides* was inhibited by N/100 acetic acid, as can be seen by the fact that it grew in N/125 concentration and that it grew in N/100 when glucose was added, while *F. oxysporum* grew well in N/100 acetic acid. N/100 formic acid was toxic to *F. trichothecioides*, while it merely inhibited growth with the other organism. The latter grew in N/125 formic acid, while *F. trichothecioides* did not grow in N/500

solution. N/100 proprionic acid was toxic to both, while both grew in N/250 solution. N/250 butyric acid was toxic to *F. trichothecioides*, while *F. oxysporum* grew in it.

TABLE IX

DRY WEIGHT (IN MILLIGRAMS) FORMED IN 12 DAYS IN VARIOUS CONCENTRATIONS OF ACID

CONCENTRATION	FUSARIUM OXYSPORUM				FUSARIUM TRICHOTHECIOIDES			
	N/1000	N/500	N/250	N/125	N/1000	N/500	N/250	N/125
Formic acid								
I	Non-weighable	Non-weighable	Non-weighable	Non-weighable	Non-weighable	None	None	None
II	Non-weighable	Non-weighable	Non-weighable	Non-weighable	Non-weighable	"	"	"
Average								
Acetic acid								
I					0.8	1.2	3.2	5.8
II					0.8	2	3.4	6.6
Average					0.8	1.6	3.3	6.2
Proprionic acid								
I	1.4	2.0	5.0	None	1.4	2.6	4.0	None
II	2.6	2.4	5.8	"	1.2	3.4	6.6	"
Average	2.0	2.2	5.4		1.3	3.0	5.3	
Butyric acid								
I	4.8	7.6	10.4	None	2.4	5.0	None	None
II	5.4	9.0	13.8	"	3.6	5.6	"	"
Average	5.1	8.3	12.1		3.0	5.3		
Oxalic acid								
I	0.4	0.4	0.4	1.2	0.8	Non-weighable	Non-weighable	Non-weighable
II	0.8	1.2	1.2	1.2	0.8	Non-weighable	Non-weighable	0.2
Average	0.6	0.8	0.8	1.2	0.8			0.1

A set of experiments was run also in which solanin in various percentages was added to glucose media. Because of the high cost of solanin, only 1 cc. solution was used in each test. The results are given in table X.

These differences, that is, greater versatility in the use of carbon sources, greater resistance to inhibition, and intoxication, may well

play an important rôle in determining the difference in behavior of these two organisms.

TABLE X

DRY WEIGHT (IN MILLIGRAMS) FORMED BY *Fusarium oxysporum* AND *F. trichothecioides* IN 6 DAYS

	Percentage solanin				
	1	2	0.25	0.226	0
Fusarium oxysporum	10.6	8.1	13.2	10	13.8
Fusarium trichothecioides	2.5	2.4	7.8	.....	9.3

DISCUSSION.—The versatility of these organisms in using various carbon sources in their metabolism is of great interest. This almost omnivorous ability to use carbon compounds, including the simplest fatty acid, the highly oxidized fatty acids, the long carbon chain acids, the alcohols, mono- and poly-hybic, glycerin and fats, the mono-, di-, and poly-saccharides, including the dextrines, starches, hemicelluloses, and true celluloses, assigns to them an important rôle in the carbon cycle, and at the same time must help render them the formidable and destructive enemies of the root crops that they are.

The methods suggested by APPEL (**2**), namely, rigid inspection of potato fields, immediate destruction of all plants that show the slightest symptoms, quarantining of non-certified seed stock, alone give promise of keeping these troubles in check. Disinfection of storage cellars and of potatoes when put into storage, together with storage at proper temperature, will help combat these diseases, especially the dry rot induced by *F. trichothecioides*.

Conclusions

1. *Fusarium tuberivorum* Wilcox and Link is the same as *Fusarium trichothecioides* Woll.

2. Both *Fusarium oxysporum* and *F. trichothecioides* can produce both tuber rot and wilt of the potato plant.

3. The wilt is induced by destruction of the root system and by clogging of the xylem elements in the stem, and is, in mild cases, marked by such symptoms as discoloration of leaves, curling and rolling of leaves, and production of aerial tubers.

4. Under field and storage conditions *Fusarium oxysporum* is more probably responsible for wilt than is *F. trichothecioides*, and the latter more responsible for tuber rotting.

5. The optimum and maximum temperatures of *Fusarium oxysporum* are higher than those of *F. trichothecioides*. *F. trichothecioides*, however, grows well at 8–10° C., while *F. oxysporum* does not. These facts may explain in part the fact that *F. oxysporum* produces more wilt than *F. trichothecioides*, and that the latter causes more tuber rot.

6. *Fusarium oxysporum* has a more rapid, superficial, and spreading habit of growth than has *F. trichothecioides*. This may be associated with a greater oxygen requirement for *F. oxysporum*, and may account for the frequenting of xylem elements by this fungus.

7. Both organisms possess a striking ability to use the most diverse carbon materials as carbon sources in their metabolism. *Fusarium oxysporum* has a greater range in its ability, and can utilize the materials more readily, although not so completely as does *F. trichothecioides*.

8. *Fusarium oxysporum* is less subject to inhibition in growth and intoxication than is *F. trichothecioides*.

9. Solanin is not toxic to either organism, although it seems to inhibit somewhat the growth of *Fusarium trichothecioides*.

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LITERATURE CITED

1. APPEL, OTTO, Untersuchungen über die Schwarzbeinigkeit und die durch Bakterien hervorgerufene Knollenfäule der Kartoffel. Arb. Biol. Abth. Land- u. Forstw. Kais. Gesund. **3**:362–432. *pl. 1. figs. 1–15.* 1903.

2. ———, Leaf roll diseases of the potato. Phytopathology **5**:139–148. 1915.

3. APPEL, O., and KREITZ, WILHELM, Untersuchungen über die Schale verschiedener Kartoffelsorten und ihre Beeinflussung durch Bodenverhältnisse, Feuchtigkeit und Düngung. Arb. Kais. Biol. Anst. Land- u. Forstw. **6**:1–27. *pls. 2. figs. 1–10.* 1908.

4. APPEL, O., and SCHLUMBERGER, OTTO, Die Blattroll Krankheit und unsere Kartoffelernten. Berlin. pp. 102. *pls. 3.* 1911. This contains a complete bibliography.

5. APPEL, O., and WOLLENWEBER, H. W., Grundlagen einer Monographie der Gattung *Fusarium* (Link). Arb. Kais. Biol. Anst. Land- u. Forstw. **8**:1–207. *pls. 4. figs. 1–2.* 1910.

6. BARY, A. DE, Die gegenwärtig-herrschende Kartoffelkrankheit, ihre Ursache, und ihre Verhütung. Eine pflanzenphysiologische Untersuchung in allgemein verständlicher Form dargestellt. Leipzig. pp. 755. 1861.

7. CARPENTER, C. W., Some potato tuber-rots caused by species of *Fusarium*. Jour. Agric. Research. Dept. Agric. **5**:no. 15. 183–209. *pls. 7.* 1915.

8. CORSAULT, J. H., Studies of the *Rhizoctonia* disease of potatoes. Phytopathology **5**:293–294. 1915.

9. DRAYTON, F. L., The *Rhizoctonia* lesions on potato stems. Phytopathology **5**:59–63. *pl. 1. fig. 1.* 1915.

10. DUGGAR, B. M., Fungous diseases of plants, with chapters on physiology, culture methods, and technique. Boston. 1909.

11. FRANK, A. B., Die Krankheiten der Pflanzen. Breslau. 1896.

12. ———, Untersuchungen über die verschiedenen Erreger der Kartoffelfäule. Ber. Deutsch. Bot. Gesells. **16**:273–280. 1898.

13. GLOYER, W. O., The efficiency of formaldehyde in the treatment of seed potatoes for *Rhizoctonia*. Bull. no. 370. N.Y. Exp. Sta. 417–431. 1913.

14. HASSELBRING, The carbon assimilation of *Penicillium*. BOT. GAZ. **45**:176–193. 1908.

15. HUMPHREY, H. B., Studies on the relation of certain species of *Fusarium* to the tomato blight of the Pacific Northwest. Bull. State Coll. Wash. Agric. Exp. Sta. no. 115. pp. 21. *pls. 5.* 1914.

16. JAMIESON, C. O., and WOLLENWEBER, H. W., An external dry rot of potato tubers caused by *Fusarium trichothecioides* Wollenw. Jour. Wash. Acad. Sci. **3**:146–152. *fig. 1.* 1912.

17. JONES, L. R., Potato diseases in Wisconsin and their control. Circ. of Information. Wis. Exp. Sta. **36**:1–10. 1912.

18. LINDAU, G., Die pflanzlichen Parasiten. Sorauer's Hand. d. Pflanzenkr. **2**:pp. 550. *figs. 62.* 1908.

19. LINK, GEO. K. K., Studies of a *Fusarium* causing dry rot of Irish potatoes. Thesis, Univ. of Neb. May 1912.

20. LINK, H. F., Observationes in ordines plantarum naturales. Mag. Gesells. Naturf. Freunde **2**:3–42. 1809.

21. ———, Species Hyphomyceten et Gymnocetum. Pars 1. 1824.

22. LONGMAN, SIBYL, The dry rot of potatoes. Jour. Linn. Soc. Bot. **39**: 120–129. *pl. 1*. 1909.
23. LOUNSBURY, C. P., Dry rot of the potato. Agric. Jour. Cape Good Hope **35**:42–48. *figs. 31*. 1909.
24. MANNS, THOS. F., The *Fusarium* blight and dry rot of the potato. Bull. no. 229. Ohio Exp. Sta. 299–336. *pls. 1–15*. 1911.
25. MASSEE, G., Textbook of plant diseases. London. 1904.
26. MORSE, W. J., and SCHAPOVALOV, M., The *Rhizoctonia* disease of potatoes. Bull. no. 230. Maine Exp. Sta. 1914.
27. ORTON, W. A., Potato wilt, leaf roll, and related diseases. Bull. no. 64. U.S. Dept. Agric. pp. 49. *pls. 16*. 1914.
28. PETHYBRIDGE, G. H., and BOWERS, G. E. H., Dry rot of the potato tuber. Econ. Proc. Roy. Dublin Soc. **1**:547–558. *pl. 48*. 1908.
29. PIZZIGONI, A., Cancrena secca ed unida delle patate. Nuovo Giov. Bot. Ital. **3**:50–53. 1896.
30. REINKE, J., and BERTHOLDT, G., Die Zersetzung der Kartoffel durch Pilze. Untersuch. Bot. Lab. Univ. Göttingen, Berlin. 1879.
31. ROLFS, F. M., Potato failures. A preliminary report. Bull. no. 70. Coll. Agric. Exp. Sta. pp. 19. *pls. 12*. 1902.
32. ———, Potato failures. A second report. Bull. no. 91. Coll. Agric. Exp. Sta. pp. 33. *pls. 5*. 1904.
33. SHERBAKOFF, C. D., Fusaria of potatoes. Memoir. Cornell Univ. Agric. Exp. Sta. **6**:90–270. *pls. 7*. 1915.
34. SMITH, E. F., Wilt disease of cotton, watermelon, and cowpea (*Neocosmospora*, n. gen.). Bull. U.S. Dept. Agric. Div. Veg. Phys. and Path. **17**: 1–26. *pls. 10*. 1899.
35. SMITH, E. F., and SWINGLE, D. B., Dry rot of potatoes due to *Fusarium oxysporum*. Bull. no. 55. Bur. Pl. Ind. U.S. Dept. Agric. pp. 64. *pls. 8. figs. 2*. 1904.
36. SORAUER, PAUL, Pflanzenkrankheiten. Berlin. 1886.
37. STEWART, F. G., and GLOYER, W. O., The injurious effect of formaldehyde gas on potato tubers. Bull. no. 369. N.Y. Agric. Exp. Sta. 385–416. *pls. 3*. 1913.
38. WEHMER, C., Über die Ursache der sogenannten "Trockenfäule" der Kartoffelknollen. Ber. Deutsch. Bot. Gesells. **14**:101–107. *figs. 3*. 1896.
39. ———, Ansteckungsversuche mit *Fusarium Solani* (Die *Fusarium* Fäule). Centralbl. Bakt. u. Par. **3**:727–743. *pls. 10, 11*. 1897.
40. WILCOX, E. M., LINK, GEO. K. K., and POOL, V. W., A dry rot of the Irish potato tuber. Research Bull. Neb. Agric. Exp. Sta. **1**: pp. 88. *pls. 28*. 1913.
41. WOLLENWEBER, H. W., Studies on the *Fusarium* problem. Phytopathology **3**:24–50. *pls. 5. fig. 1*. 1913.
42. ———, Pilzparasitäre Welke-Krankheiten der Kulturpflanzen. Ber. Deutsch. Bot. Gesells. **31**:17–34. 1913.

AERIFEROUS TISSUE IN WILLOW GALLS

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(WITH PLATES II–IV AND FIVE FIGURES)

COSENS (2) has demonstrated recently that in certain willow galls there is a production of aeriferous tissue in regions where it is not present normally. This investigation has been undertaken for the purpose of explaining the unexpected appearance of this tissue. Many different species of *Salix* and *Populus* were examined in order to determine the normal distribution of the tissue in their various organs. As an important adjunct of the work experiments have been conducted for the purpose of observing the influence of certain factors on its production, such, for example, as temperature, water, nutrient solutions, and light.

Stated in general, intercellular spaces are found in the parenchymatous tissues of nearly all plants. These usually remain small and inconspicuous, although connected with one another in such a way as to form a continuous gas-containing system of cavities. In many plants, however, especially hydrophytes, these spaces are so large and conspicuous and formed on such a regular plan as to impart the well known characteristic appearance to the areas in which they occur. Such a typical tissue is designated here aeriferous tissue.

Concerning the occurrence of this tissue in certain histological regions of *Salix* when under abnormal stimulation, two explanations present themselves: (1) that the tissue is an "environmental" modification, produced by the action of the plant protoplasm in direct response to certain conditions of the environment; (2) that the tissue is a distinct type, probably of wider distribution in the ancestral forms of the group, but now absent in certain regions of the modern species, since the power to produce it is dormant, but reinstated when these tissues are subjected to suitable stimulation. One example will suffice to illustrate the line along which attention has been directed. In many aquatic plants there are two types of leaves; for example, in *Proserpinaca palustris* L. the leaves on the

submerged portions are divided, while those on the aerial portions are entire. Explanations, on experimental grounds, have been offered by various workers, notably McCallum, Burns, and Goebel, to account for this phenomenon of heterophylly.

McCallum (4) conducted a series of experiments with *Proserpinaca palustris* L., in which both submerged and aerial plants were grown under various degrees of illumination, and found that the type of leaf developed was entirely independent of the light relations. Further experiments were performed in which plants were kept in a perfectly saturated atmosphere containing normal amounts of carbon dioxide and oxygen, and it was observed that the water form of leaf persisted. It was then assumed that the essential factor common to moist air and water was inhibition of transpiration, and confirmatory experiments from this standpoint were carried out, in which transpiration was induced in submerged plants by high osmotic pressure. The conclusion arrived at was that the aerial type of leaf is developed, even under water, if sufficient moisture is withdrawn from the protoplasm.

From the preceding experiments the conclusion deduced was that the only factor, constant in all cases where the water form of leaf developed, was the checking of transpiration and constant increase in the amount of water in the protoplasm, while those chemical and physical conditions, resulting from partial withdrawal of water, are associated with the aerial type. The main point is that the modification in the form of the leaf was construed as environmental, that the form of the leaf is especially plastic under the influence of the transpiration factor.

Burns (1) studied the same plant and arrived at conclusions entirely different from those formed by McCallum. He experimented with cuttings and seedlings, and found that in the case of the former the disturbance of the vegetative activity caused a change that might be accompanied by a reversion, but concluded that environmental factors did not determine, in McCallum's sense, the type of leaf produced. A verification of this was obtained from the production of seedlings under varied conditions of light, temperature, moisture, etc. He also found in the case of plants grown from seed that the first leaves to appear were

always divided, and that the entire leaves were of later growth. From further experiments he obtained the following data. All stems, regardless of external conditions, produced the water type in the autumn. Stems from which the vegetative points were removed in June threw out side branches with the water type of leaf, while normal plants under the same external conditions were producing the land type. In summer all plants, whether in water or air, developed the aerial form of leaf. Also, at the time of flowering only entire leaves were found. The change from the water to the land type took place earlier on strongly growing than on weak stems.

From his experimental results BURNS concluded that there are two forms of leaves, an adult and a juvenile, and that the production of these is not controlled by any one or more definite factors of the environment, such as light, moisture, temperature, etc. But under favorable vegetative conditions the adult form, the entire leaf, is produced, while under unfavorable the juvenile divided leaf is developed. Further, a reversion to the primitive or divided type may be associated with unsuitable vegetative conditions.

GOEBEL (3) experimented for several years with plants that exhibited the phenomenon of heterophylly, and his views fully confirm those of BURNS. He summarizes his conclusions concerning *Proserpinaca* as follows:

> Es ist klar, dass die Wasserumgebung nicht die Ursache der Blatteilung ist. Ebensowenig hängt sie ab vom Licht, der Temperatur, dem Gasgehalt des Wassers und dem Kontaktreiz als Solchem. Die einzige Folgerung, die sich aus meinen Versuchen ableiten lässt, scheint die zu sein, dass Proserpinaca palustris zwei Formen hat, eine "Folgeform" und eine "Jugendform." Unter guten vegetativen Bedingungen hat sie das Bestreben, die Folgeform mit den ganzen Blättern, Blüte und Frucht hervorzubringen, unter ungünstigen Vegetationsbedingungen hat die Pflanze das Bestreben, die Jugendform mit dem geteilten Blatt zu bilden.

In view of the satisfactory results obtained from investigations on such morphological features, it seems entirely reasonable to hope that the anatomical aberrations occurring in galls may be capable of explanation by pursuing similar lines of inquiry and by making use of the anatomical principles unfolded by SCOTT, JEFFREY, and others in recent years. Several of these aberrations have been

studied by the senior author; in this paper that of aeriferous tissue only is dealt with in detail.

Distribution of aeriferous tissue in galls

Aeriferous tissue is abnormally present in galls produced by certain species of at least two distinct orders of insects. Thus it has

1 2

FIGS. 1, 2.—Fig. 1, Gall of *Rhabdophaga triticoides* Walsh on *Salix cordata* Muhl.; fig. 2, Galls produced by *Rhabdophaga triticoides* Walsh.

been located in the dipterous galls *Rhabdophaga triticoides* Walsh and *R. strobiloides* Walsh, stem galls on *Salix cordata* Muhl., and *Phytophaga rigidae* O.S. on the stem of *Salix discolor* Muhl. It also occurs in the hymenopterous gall *Pontania pomum* Walsh, a leaf gall on *Salix cordata* Muhl.

The stem gall *Rhabdophaga triticoides* Walsh is usually terminal or nearly so (text fig. 1), but sometimes occurs at varying distances

from the tip of the stem; two or three galls occasionally are spaced along a branch and separated by sections of normal stem, as shown in text fig. 2. The gall, especially when of the terminal type, bears a remote resemblance to a head of wheat. It is produced by a very marked shortening of the stem of the host, accompanied by a decided increase in its diameter. The contraction of the infected

3 4

FIGS. 3, 4.—Fig. 3, Gall produced by *Rhabdophaga strobiloides* Walsh on *Salix cordata* Muhl.; fig. 4, Gall of *Phytophaga rigidae* O.S. on *Salix discolor* Muhl.

stem is so pronounced that the lateral buds, which would normally occupy 25–30 cm. of the stem, are crowded into a space of 4–5 cm. As the characteristic alignment of the lateral buds is approximately maintained on the abnormal part of the stem, there are 5 fairly well defined rows of buds on the gall. In each of these buds a larval chamber is located.

Practically the entire pith and the cortex of this gall are composed of aeriferous tissue. It is absent only immediately around the zonal areas of the larval chambers, and in these regions it can be seen to have been obliterated by compression. The tissue is of

the most typically aeriferous type, consisting of polygonal spaces bounded by strands seldom more than one cell in width. The distribution and character of this tissue can be seen in figs. 1 and 2.

The gall *Rhabdophaga strobiloides* Walsh (text fig. 3) is invariably terminal, since the solitary larva occupies the tip of the growing point, and thus prevents any elongation of the stem past the infected area. The condensation of the stem axis that results is, in this case also, the chief gall-producing factor, the top-shaped mass of aborted leaves representing the normal leafage of nearly a meter of stem. The entire cortex of the receptacle of this gall is composed of aeriferous tissue. It commences to appear in the pith, immediately beneath the compact tissues of the gall zones, and extends to a considerable distance from the point of infection (fig. 4.)

FIG. 5.—Galls on *Salix cordata* Muhl. produced by *Pontania pomum* Walsh.

In contrast with the two preceding species, a marked condensation of the stem of the host does not enter into the production of the gall *Phytophaga rigidae* O.S. (text fig. 4). This is apparent from the normal spacing of the buds that persist on the exterior of the gall. The pith of the stem in this form is occupied by the larva of the producer, and only small areas of parenchyma remain outside of the protective sheath of the gall, but in these aeriferous tissue can be detected. The cortex, which is very thick, consists entirely of the same tissue (fig. 6). Aeriferous tissue is also developed far in excess of the normal in the cauline stelar gaps comprised within the gall. A section of such a stem gap is shown in fig. 3.

The normal occurrence of aeriferous tissue in the Salicales will be discussed in detail later, but it may be stated here that, while it occurs in the stem pith of the three galls just described, it is not present in the corresponding regions of normal *Salix*.

The well known spherical gall produced by the sawfly *Pontania pomum* Walsh on the leaves of *Salix cordata* Muhl. (text fig. 5) furnishes another example of aeriferous tissue in association with a gall-producing stimulus. In its earliest stages the gall consists of a singularly compact tissue (COSENS **2**), but gradually the aeriferous characters become evident, until almost the entire mass of the adult gall consists of tissue which is thus differentiated. Fig. 5 is a typical section from such a mature gall.

Distribution of aeriferous tissue in normal plants

For the purpose of determining the distribution of this tissue in normal plants, the following species of Salicaceae were examined: *Salix fragilis* L., *S. cordata* Muhl., *S. discolor* Muhl., *S. humilis* Marsh., *S. longifolia* Muhl., *S. lucida* Muhl., *S. nigra* Marsh., *S. petiolaris* Sm., *S. purpurea* L., *S. rostrata* Richards, *S. serissima* Fernald, *Populus balsamifera* L., *P. deltoides* Marsh., *P. grandidentata* Michx., *P. nigra* L., *P. tremuloides* Michx.

CORTEX.—At first sight the cortex, both primary and secondary, of all these species appears to consist of a compact tissue, but on further examination a well defined aeriferous tissue was found in the primary cortex of every one. It can be seen in sections taken from the apex of the stem and for some distance back (fig. 10), but in older regions it is destroyed by compression. The amount formed varies somewhat in different species. Thus in *Salix nigra*, *S. cordata*, and *S. fragilis* the entire tissue forming the primary cortex is aeriferous; the meshes are relatively large, and the walls inclosing them are as a rule but a single cell in thickness. In *S. rostrata* and *S. petiolaris* it is especially well differentiated at the angles of the stem; at some levels it is confined to these regions.

Among the poplars the largest amount of this tissue is possibly to be found in *P. nigra* and *P. tremuloides*. In the former it is very pronounced, for the cortex is an entire lattice work of it, and the air spaces are large and bounded by strands that vary from one to three cells in width. The meshes in *P. tremuloides* are likewise regular and clearly defined, but they attain their greatest dimensions at the angles of the stem. *P. balsamifera* is peculiar in that they are most abundant and characteristic immediately around the

leaf traces, especially in close approximation to the bundles. Indeed, at the nodal levels the entire cortex consists of aeriferous tissue. It is less apparent in *P. grandidentata* and *P. deltoides*, for, though present, it is not as abundant nor as regularly defined as in the other species. Incidentally it may be stated that wherever this tissue occurs in the cortex of the normal plant it is much more abundant and better developed in corresponding parts of stem galls. This also applies to *Salix*.

PITH.—The pith is distinctive and important. It has been noted that the pith in the galls cited is occupied by a very pronounced aeriferous tissue, but this is not the case with the normal pith of any species of willow except in the reproductive axis. In every species examined it is a compact tissue without any indication of other than small, ordinary, inconspicuous intercellular air spaces. The pith illustrated in figs. 8 or 10, for example (a normal pith), stands out in striking contrast to the pith of the gall of fig. 2. It is especially noteworthy that in the latter case the medullary and cortical tissues are identical, both in their conformation and in the size and shape of their cellular constituents, while in the normal, the cells of the pith are much larger and closely packed. The air spaces of the aeriferous tissue do not result as an indefinite elongation of the cells, as in certain oak-leaf galls, but are the result of cell divisions which follow a definite law with regard to their polarity. The compact normal pith and the open abnormal pith appear to be fundamentally different types on this basis and on the basis of the peculiarities in the distribution of the latter to be noted.

Fig. 7 is a photograph of a normal stem of *Populus balsamifera* taken from a new shoot, and it illustrates the difference manifested by *Populus* in comparison with *Salix*. In all poplars investigated the plan of organization of the pith is that of an aeriferous tissue, though lacking the regularity found in the cortex. Were it established, as some believe, that *Populus* is the more primitive genus, this phenomenon would be important in explaining the aeriferous tissue in the pith of the galls of *Salix*.

NODE.—We have been taught to regard the node as a conservative part of the stem, one in which discarded tissues may still persist,

so that much interest attaches to an examination of this region. It is at the node more than elsewhere in the stem that the aeriferous tissue is most abundant and characteristic in both willows and poplars. Thus in *P. balsamifera* it is particularly abundant immediately around the leaf traces, especially in close approximation to the bundle. The branch gaps likewise are occupied by this tissue, in the case of *Salix* extending well into the pith (fig. 8). In stem galls, for example, *Phytophaga rigidae* O.S. on *Salix rostrata*, this feature is always very pronounced; but most significant of all, the pith at the bases of the branches is typical aeriferous tissue. Fig. 9 illustrates this feature clearly, and is interesting in comparison with fig. 10; both are from *Salix lucida*, the former from the base of a branch, the latter from an internode.

LEAF.—The petiole is sometimes a vestige carrier, so that it is of interest to note that aeriferous tissue has been located here in several species of *Salix* and *Populus*, for example, *Populus balsamifera*, *Salix humilis*, *S. fragilis*, and *S. cordata*. As a rule it is confined to the part of the petiole adjacent to the stem. Elsewhere the ground tissue is of the compact type. In the blade there is no indication of it, as the palisade layer and spongy parenchyma are of the ordinary type. Yet in the leaf gall *Pontania pomum* on *Salix cordata*, practically all of the ground tissue is aeriferous.

SEEDLINGS.—The seedlings of *Salix serissima* Fernald only were examined. These were obtained by sprouting the seeds in a moist chamber between layers of filter paper in an incubator, and then pricking the young seedlings into small fern pans. Different stages were examined, but in none was aeriferous tissue found in the pith. It is present in the cortex.

ROOT.—The roots of the Salicaceae are devoid of a pith. The primary cortex, however, was examined in the roots of seedling plants of *Salix serissima*, and in young roots of *Populus balsamifera* and *P. nigra*, and was found to consist in part of a well differentiated aeriferous tissue.

INFLORESCENCE.—No part of the plant is considered more important as a retainer of vestigial tissue than the reproductive axis. It is of especial interest to find, therefore, that aeriferous tissue is developed and often beautifully typical and abundant in

the pith as well as in the cortex in the reproductive axes of all of the species of *Salix* and *Populus* that were investigated. This is especially noteworthy in the case of *Salix*, because here and at the bases of the branches alone is pith of this type to be found. Figs. 11, 12, 13, and 14 are photographs of the pith of sections cut from the catkins of *Salix purpurea*, *S. humilis*, and *S. cordata*. Figs. 11 and 12 are of *S. purpurea* at different stages of development, the former from a young catkin, the latter from a mature one. This tissue is sometimes more abundant and typical in the pith of the staminate catkin, but a striking similarity exists in the form and distribution of the tissue in the two catkins of the same species. An examination of both was made in the case of *Salix alba*, *S. lucida*, and *S. longifolia*. In *S. rostrata*, *S. cordata*, and *S. nigra*, the aeriferous tissue of the pith is more clearly defined than in the cortex, but is present in both. The reverse is true in *S. lucida*, while in *S. discolor* there is no greater development in the one region than the other. The pith in the catkin of *S. petiolaris* is peculiar because it consists of a large-meshed aeriferous tissue, and often there are but two or three large spaces bounded by slender lines of cells.

Influence of environment on occurrence of aeriferous tissue

For the purpose of ascertaining to what extent the production of aeriferous tissue in the Salicales is dependent on external factors, several experiments were conducted in the growing of plants under conditions in which certain components of the environment could be controlled. Cuttings from various species were grown in culture solutions, in water, some totally immersed, and in soil. The effect produced by the variation of the illumination and temperature was also noted in a number of species. The following list comprises the more important experiments.

LIGHT RELATIONS.—Cuttings from *Salix nigra* Marsh., *Populus nigra* L., *Populus balsamifera* L., and others were sprouted in pots of earth. In the conservatory some of these were placed in a situation to receive the maximum light, others in a more sheltered position, and finally several in a light-proof chamber. The only effect noted was in the case of the sprouts grown in the dark. In those experiments the stems produced were flattened, and the tissues,

which were greatly compressed, contained many parenchyma cells of particularly large size, but no aeriferous tissue.

TEMPERATURE RELATIONS.—Cuttings were sprouted as in the preceding series, but the experiments were conducted at several different temperatures. With the exception of an increase in the rate of growth, tissue effects were not produced.

INHIBITION OF TRANSPIRATION.—Cuttings from various species of *Salix* and *Populus* were weighted down in tall cylinders containing water and allowed to sprout when completely submerged. Some of these cuttings were placed in bright sunlight and others in more shaded situations. Sections were taken from the newly produced shoots at different levels, as the growing point, the base of the sprout, near its origin from the main stem, and also from shoots that had just emerged from the water. In this series the most marked effect found was the flattening of the stems and the compression of the tissues proportional to the depth at which they were grown. In no case was there any increase in the amount of aeriferous tissue produced; indeed, in the sprouts produced on cuttings from *Salix nigra* Marsh., the tissue was entirely lacking.

CULTURE SOLUTIONS.—Cuttings were sprouted in various nutrient solutions, including all of the formulae recommended by MACDOUGAL (5), and the results were checked by shoots produced in ordinary water. In the sprouts produced on *Salix* cuttings in nutrient solutions aeriferous tissue was not developed in the pith. While it was invariably present in the pith of the *Populus* shoots, it was not increased in amount beyond the normal production.

The results of all the experiments seem to justify the conclusion that the stimulus to the development of aeriferous tissue in the Salicales is not involved in light, in temperature relations, in the nutrient conditions imposed by the conditions of our experiments, or in checking of transpiration.

Discussion

As already stated, two theories present themselves in explanation of the unexpected appearance of aeriferous tissue in certain regions of insect galls where it is normally absent. The tissue may be regarded as a direct effect of the action of certain environmental

factors on the protoplasm of the host; or it may be considered a hereditary tissue that has been reinstated owing to the awakening of latent characteristics in the protoplasm of the infected plant, on account of unusual conditions associated with the presence of the gall producer.

Concerning the alteration by experiment of the environment of the host plant, all attempts were unsuccessful either to increase the amount of aeriferous tissue normally present or to cause its development in regions where it is not found under normal conditions. Although the inhibition of transpiration is commonly regarded as an important factor in the production of this tissue, it was not increased to the smallest extent in the *Salix* sprouts developed under conditions of reduced transpiration. Indeed, if these experiments had given positive results, it would still be necessary to show that the conditions presented by galls are those of restricted transpiration. This is clearly not the case in *Rhabdophaga triticoides* Walsh, the gall in which the development of the tissue is the most pronounced and its characteristics the most typical. As stated previously, the lateral buds that contain the larval producers of this gall are situated on a length of stem, the internodes of which are shortened to a very marked degree. The occupation of the buds by the larvae, however, has not checked the development of the leaves in the axils of which the buds are produced. The galls shown in text figs. 1 and 2 were collected after the leaves had fallen. This results in the leaf area of the abnormal part of the stem being increased far beyond the normal leafage, a condition that we can scarcely associate with reduced transpiration. Thus the direct evidence from experiments leads us to believe that environmental factors of light, temperature, moisture, etc., are not directly operative in the production of aeriferous tissue.

The anatomical structure of the normal stems of the Salicales strongly favors the same view by furnishing facts apparently explainable only on the supposition that we are here dealing with a true heritable tissue. In accordance with the general view concerning the retention of ancestral characteristics, certain regions of plants are regarded as more tenacious of these structures than others. For example, it is commonly recognized

that they may persist in the reproductive axes, petioles, leaf or branch traces, and roots. The tissue under consideration must be regarded as primitive in accordance with this general principle. It occurs in the pith and cortex of the reproductive axes of all species of *Populus* and *Salix*. It is found in the pith at the bases of the branches of the stem of *Populus* and *Salix*, and only in that region in the case of the latter genus. Further evidence in support of the same view is furnished by its presence in the branch gaps, its occurrence in petioles and the vicinity of stem and leaf traces, and in the cortex of the root. This prominent development of the tissue in the vestigial carriers of both *Populus* and *Salix*, taken in conjunction with our experimental results, presents strong evidence in favor of the theory that the aeriferous tissue in the Salicales is of ancestral type.

The power of the gall stimulus to arouse latent properties in the protoplasm of the host, expressed by its ability to produce an aeriferous tissue not normally present in *Salix*, is also exemplified in the case of glandular tissue and trichomes in certain galls on other plants. It may be stated as a general principle that glands, when they are present normally in a tissue of the host, are always more plentiful or larger in the gall originating from that tissue. A striking example (COSENS **2**) of this is furnished by the pithy spherical stem gall produced on *Solidago canadensis* L. by *Eurosta solidaginis* Fitch. But in some cases glands are produced in galls on parts of the host normally glandless. Thus, they are plentiful in the stem gall *Neolasioptera perfoliata* Felt on *Eupatorium perfoliatum* L., but are not present in the same location in the normal plant. In fact, they were found in normal plants in the transitional region between stem and root only, both in this species and in *E. urticaefolium* Reichard.

Trichomes exemplify the same principle. When the gall produces unusual trichomes, the abnormal forms can almost invariably be found on the reproductive axes of the host. Thus, while the normal hairs on the leaf of *Quercus macrocarpa* Michx. are of the stellate type, those of the reproductive axes are acicular, corresponding exactly to those composing the pubescence of the leaf gall *Eriophyes querci* Garman on this host. Also, the convoluted type

of hair present on the *Acarina* dimple gall on the leaves of *Acer Negundo* L. are exactly reproduced by the form occurring on the reproductive axes, although the normal leaf hair is straight.

An almost dormant characteristic has also been aroused in *Rosa blanda* Ait. by the cynipid producer *Rhodites multispinosus* Gillette (COSENS 2). The gall produced is always exceedingly spiny, although the stem of the host from which it originates is usually unarmed. But it is worthy of note that the production of spines is a marked character of other species of the genus. Doubtless further investigations will show that examples of this kind can be almost indefinitely multiplied.

The reinstatement in a gall of vestigial characteristics of the plant has an important bearing on the question of gall formation. The producer has long been recognized as exercising a directive control over the activities of the protoplasm of the host, but these examples of the rehabilitation of dormant characters show that the forces operative in gall formation are of wider scope. Under these conditions unexpected structures and unusual combinations may well be produced, and in the interpretation of the morphology of any gall it becomes necessary to discriminate carefully between these two classes of organs and tissues, that is, between those that are simply environmental modifications of the normal and those that are vestigial or in use in other parts only of the plant. Incidentally it may be added that there remains no authentic instance of any organ or tissue in a gall that is new, ontogenetically or phylogenetically, to the host.

This investigation was carried on at the Botanical Laboratories of the University of Toronto, and the authors wish to acknowledge their indebtedness to Professor J. H. FAULL for invaluable criticism throughout the whole course of the work.

UNIVERSITY OF TORONTO

LITERATURE CITED

1. BURNS, GEO. P., Heterophylly in *Proserpinaca palustris*. Ann. Botany **18**: 579–587. *pl. 38*. 1904.
2. COSENS, A., A contribution to the morphology and biology of insect galls. Trans. Canadian Inst. **9**: 297–387. *pls. 13*. 1912.

3. GOEBEL, K., Einleitung in die experimentelle Morphologie der Pflanzen. Leipzig: Teubner. 1908.
4. MCCALLUM, W. B., On the nature of stimulus causing the change of form and structure in *Proserpinaca palustris*. BOT. GAZ. **34**:93–108. *figs. 10.* 1902.
5. MACDOUGAL, D. T., Practical textbook of plant physiology. New York: Longmans. 1901.

EXPLANATION OF PLATES II–IV

PLATE II

FIG. 1.—*Rhabdophaga triticoides* Walsh on stem of *Salix cordata* Muhl.: transverse section showing general arrangement of larval chambers and distribution of aeriferous tissue throughout the cortex and pith of the gall; ×10.

FIG. 2.—*Rhabdophaga triticoides* Walsh on stem of *Salix cordata* Muhl.: transverse section in which is shown the typical character of the aeriferous tissue produced under the stimulus of the gall producer; ×50.

FIG. 3.—*Phytophaga rigidae* O.S. on *Salix discolor* Muhl.: a stem gap which shows aeriferous tissue produced in excess of normal amount; ×90.

FIG. 4.—*Rhabdophaga strobiloides* Walsh on stem of *Salix cordata* Muhl.: a longitudinal section showing aeriferous tissue produced in pith of gall axis; ×25.

PLATE III

FIG. 5.—*Pontania pomum* Walsh on *Salix cordata* Muhl.: section of a nearly full-grown gall in which aeriferous tissue is produced throughout its mass; ×18.

FIG. 6.—*Phytophaga rigidae* O.S. on *Salix discolor* Muhl.: gall chamber includes all the pith area of the stem; aeriferous tissue of the characteristic type occupies whole of cortical region of the gall; ×10.

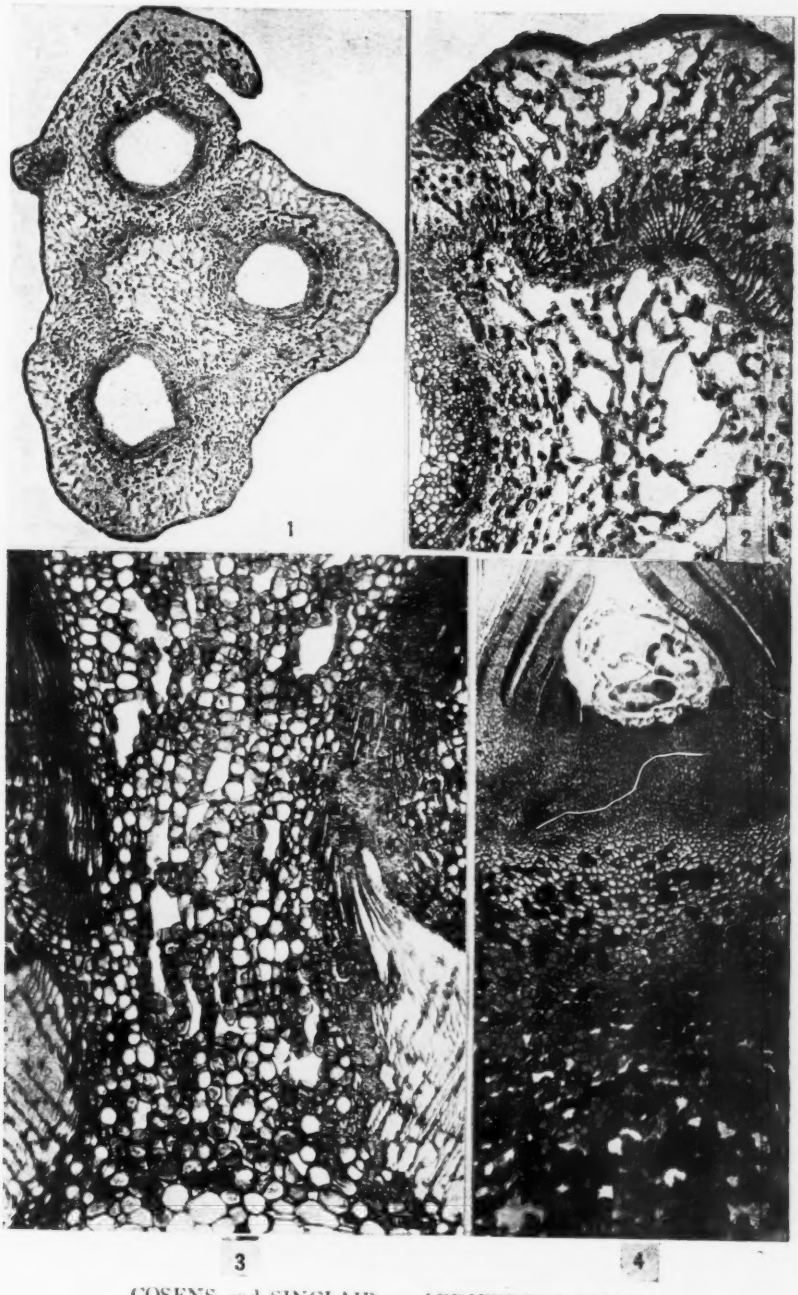
FIG. 7.—*Populus balsamifera* L.: transverse section of stem pith in which aeriferous tissue is normally present; ×80.

FIG. 8.—*Salix humilis* Marsh.: section of normal stem at level of a stem gap; it illustrates the compact nature of the *Salix* pith and the differentiation of aeriferous tissue in the stem gaps; ×65.

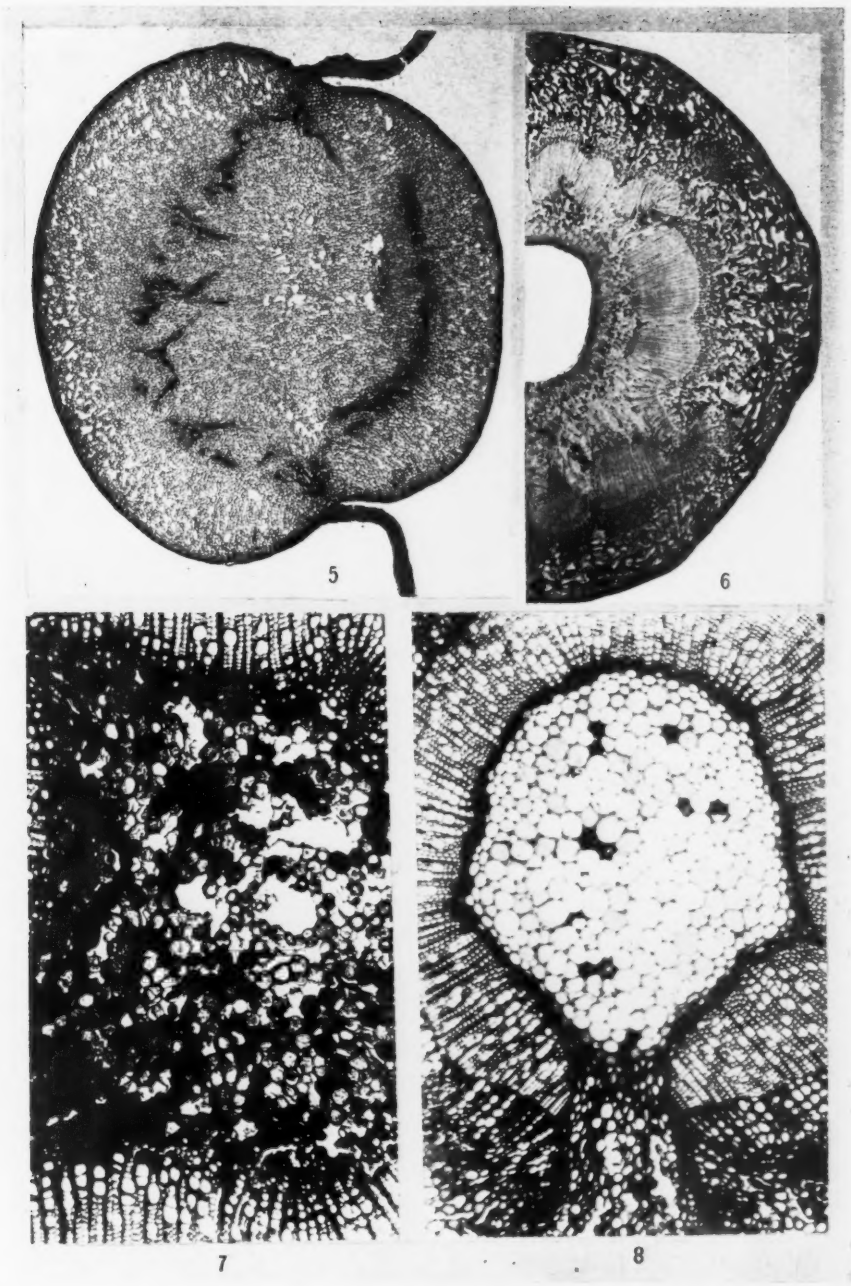
PLATE IV

FIG. 9.—*Salix lucida* Muhl.: section through base of a normal stem, showing aeriferous tissue produced in the pith; ×60.

FIG. 10.—*Salix lucida* Muhl.: transverse section of normal stem a short distance behind the growing point; aeriferous tissue constitutes the entire cortex, but is completely absent from the pith; this compact nature of the pith is typical for all species of *Salix* except in region of bases of branches as illustrated in preceding figure; ×12.



COSENS and SINCLAIR on AERIFEROUS TISSUE



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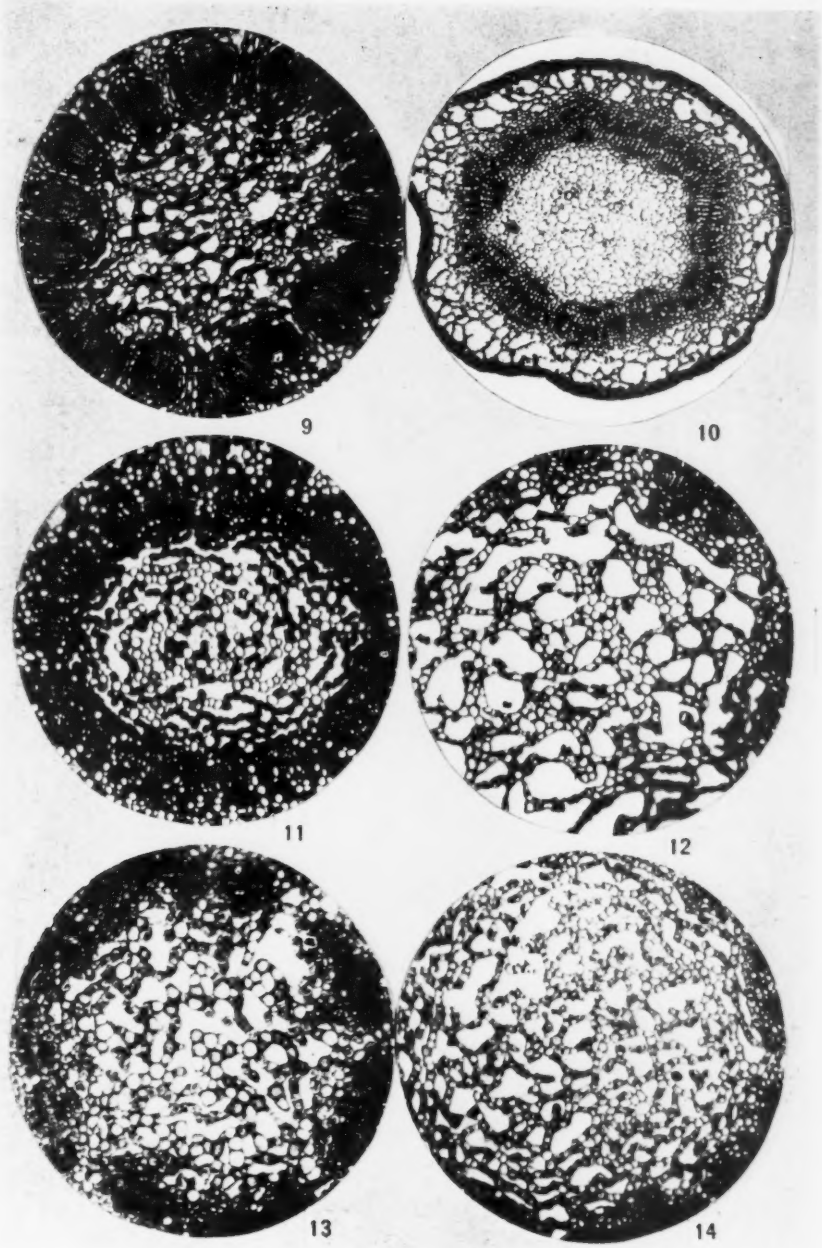

COSENS and SINCLAIR on AERIFEROUS TISSUE

FIG. 11.—*Salix purpurea* L.: section across the male reproductive axis when the catkin is just emerging from the bud; aeriferous tissue is clearly differentiated in the pith; ×70.

FIG. 12.—Same as preceding except that the catkin was sectioned at a later stage; aeriferous tissue has opened out, making more apparent the air spaces and the separating strands of cells; ×70.

FIG. 13.—*Salix humilis* Marsh.: showing aeriferous tissue developed in the pith of the male reproductive axis; ×70.

FIG. 14.—*Salix cordata* Muhl.: aeriferous tissue in the pith of the male reproductive axis; a comparison with fig. 2 shows similarity of this tissue with that incited by gall stimulus in the stem pith of this species of *Salix;* ×70.

CHONDROMYCES THAXTERI, A NEW MYXOBACTERIUM

J. H. FAULL

(WITH PLATES V AND VI)

The Myxobacteriaceae constitute an extremely interesting assemblage of forms, because of their apparent relationship to the bacteria on the one hand, and to the slime molds, particularly the Acrasieae, on the other hand. The individual plants are bacteria-like rods in all cases, and after a vegetative period these swarm together to organize a definitely shaped pseudo-fructification, without, except in the one genus *Myxococcus*, undergoing any marked morphological changes themselves. These fructifications are not comparable to the more or less heaped up colonies characteristic of certain bacteria, but, as THAXTER (9) has pointed out, are strictly comparable to the "fructifications" of the Acrasieae, the only other group in which there exists "a similar concerted action of aggregates of individuals toward a definite end, namely, the production of a more or less highly differentiated resting state." Whether they are to be regarded as a family of bacteria or as a division of the Myxomycetes, close to, if not included within the Acrasieae, remains undecided. BAUR (1) and QUEHL (4) and more recently PINOY (3) incline strongly to the former view; VAHLE (10) and others incline to the latter view.

Of the fewer than 30 known bona fide species, distributed among 3 genera, 11 have been referred to the complex and variable genus *Chondromyces*. The one described in this paper adds another, *Chondromyces Thaxteri*, n. sp., in some respects the most remarkable of all. This form appeared in small quantity on deer dung from Algonquin Park Forest Reserve, Ontario, and was successfully cultured for more than two years, or until the identity of the species was established, when it was allowed to die out.

The fructifications, or more properly pseudo-fructifications, are ordinarily yellowish, but may vary from flesh-colored to reddish

orange, in this as in other respects showing great variability, and ranging 250–750 μ in height, averaging about 350 μ. They are usually simply stalked, but the stalks may fork or even branch more than once, as is shown in fig. 5. The stalks are broad-based, narrowing slightly toward the apex, and yellowish in color. Within their gelatinous matrix are considerable numbers of rods, especially in the lower part. It may be noted also that many members of the colony contributing to the fructification are left on the medium in the immediate neighborhood.

At the tip of the stalk a single head of rods may be found, but more frequently several are formed, resulting from a lobing or branching of the mass that has attained this level, an example of which is represented in fig. 3. These lobes or heads may be sessile or short-stalked. This habit calls to mind *C. pediculatus* Thaxt., in which species the lobes at once ripen as the ultimate cysts, and *C. catenulatus* Thaxt., in which, however, the lobes first elongate and then contract at intervals, due to secondary movements of the rods, giving rise to chains of cysts. But development goes further here. A striking phenomenon next takes place, namely, a heaping up of the rods in radially arranged masses over the entire surface of each head, in the form of elongated cones (figs. 7, 11) or, more rarely, in the form of cylinders (figs. 4, 12), likewise of comparatively great length. In the first instance the apices of the cones are often attenuated to such an extent as to be tipped with but single rods. The surface of the now bristling head is directly invested closely by a yellowish transparent membrane, a secretion from the rods.

The cylindrical form has its counterpart in *C. crocatus* B. and C., in which, however, the cylinders become sharply abstricted at the base, and mature as cysts, when ripe falling away from the cystophore and from one another at the slightest touch; and similarly the conical form, which is the more frequent of the two, recalls *C. apiculatus* Thaxt., possibly its most closely related species. In *C. apiculatus* the cones develop into cysts as do the cylinders in *C. crocatus*, but this is first initiated by a secondary migration of the rods to the centers of the cones, the covering membrane simply shrivelling at the bases and apices.

In *C. Thaxteri* the purpose of the cone and cylinder formations is not evident, for immediately succeeding the completion of the envelope there is a migration of the rods back to the center of the head, within which they form a smooth, more or less spherical mass, the membranes of the protuberances persisting as somewhat shrivelled and twisted or striated appendages, containing no rods except such occasional stragglers as may happen to have adhered to their inner surfaces.

The bristly heads, without further morphological changes, then mature as cysts, which drop off almost as lightly as do the spore-like cysts of *C. crocatus*. Figs. 3, 4 or 7, 9, and 1 represent in sequence the stages that have been described. The course of development just outlined is entirely independent of variations in moisture, food supply, light, and temperature, so far as I have ever observed; always the formation of cones or cylinders on the surfaces of the heads, and their subsequent abandonment, the rods retreating over the route along which they came. The ripening of the head is completed by a movement of rods away from the short stalk, and a shrivelling or contraction of the membrane at that point, which thus explains in part the mode of dehiscence (fig. 1).

The number of cysts, as already indicated, varies from one to several, usually 3–4, but sometimes there may be as many as 20–30. Not infrequently fusions take place in the early stages between neighboring developing heads, as shown in fig. 2. When mature, the cysts are spherical or more commonly depressed spheres or thick disks, and average about 140 μ in extreme width, though varying from 65 to 165 μ. These measurements include the appendages, which vary from 30 to 15 μ in length, and from 10 to 22 μ in breadth at their bases.

On germination of the cysts the rods swarm out through the basal scar of the membrane, leaving the bristly empty husk behind. Fig. 14 indicates the way in which this process takes place, and also shows that the appendages on the membrane have been septated off in no way by an inner secondary membrane.

Attention has already been called to the many variations exhibited by *Chondromyces Thaxteri*, a phenomenon shared to some extent by other species; variations in color, striking variations

in every dimension of the fructification, variations in the branching of the stem, and in the number and size of the cysts and their appendages. Compare, for example, figs. 1, 2, 5, 8, 9, and 10. Many species might easily be based on a series of mounts made from a single culture. There is no doubt, of course, that these are simple fluctuating variations of very wide range. It is also almost certain, although not demonstrated, that many of these are due to environment; the local differences of food supply and moisture on the substrata on which they grow cannot be inconsiderable; but this does not explain all.

The life history was followed carefully throughout in its main features, and was found to conform closely to the account given by THAXTER for *C. crocatus*. Confirmation is given also of the fact that the contents of the rods are characterized by deeply staining masses, especially just before and during the resting stage, one to three or four being readily demonstrable. Eosin differentiates them especially well in the cystophores, where the imbedded rods, stained deep pink, stand out in striking contrast to the yellowish translucent matrix. This stain also brings out the uneven distribution of the rods in the cystophore; often there is a thin outer zone almost free from them, and, too, they are much more abundant toward the base than elsewhere, gradually diminishing in number toward the apex. The contents of the cysts stain deeply; the envelope remains unstained, chitinous yellow in appearance, showing for the cysts at all events that the color is not restricted to the rods, as stated by BAUR.

Many attempts were made to secure pure cultures, and a variety of media was employed. The only one that yielded tolerable results was the natural substratum of the organism. Moreover, even on this it was impossible to secure good results if the cysts alone were planted. There was always an admixture in flourishing cultures. What relation the impurities bore to the *Chondromyces* I did not determine. It may be a case of succession of floras, in which the ground must first be prepared for the *Myxobacterium*. So far as I have been able to learn, the same feature is true of other species that have been cultivated. PINOY (3) has recently investigated this feature of *Chondromyces crocatus*, and

he announces that a *sine qua non* condition for obtaining a complete development of the fructification is the presence of a special bacterium, a *Micrococcus* related to *M. latens*. He demonstrated this by first isolating the coccus and then planting it alongside rods of *Chondromyces* on the surface of the same sterilized medium. It was only where the resultant colonies grew together and mingled that perfect fructifications of *Chondromyces* were formed, although the relation does not appear to be lichenicolous in character. He affirms that if the *Micrococcus* be present, normal cultures can be obtained on many different kinds of media. I know of no further observations on this subject. ZEDERBAUER (11) cannot be quoted, because he was not working with Myxobacteriaceae as he supposed. In my own cultures a filamentous fungus with septate hyphae was constantly present in small amount, and not infrequently the threads of this fungus are imbedded in the cystophore, although never in the cysts. But ample observations make certain that whatever rôle it plays, if any, it is not a necessary constituent of the fructification at any stage.

The species of *Chondromyces* that have been so far described constitute a rather close evolutionary series; and the outstanding character in which the evolutionary forces are manifested is fundamentally not one of form, size, or color, but of movement. Thus we may begin with a form like *C. muscorum* Thaxt., in which single, simple, unstalked cysts are formed. In *C. serpens* Thaxt. and *C. lichenicolus* Thaxt. there is a movement in a horizontal direction, resulting in sessile, elongated, and often confluent, coil-like cysts. The primary movement is vertical in *C. gracilipes* Thaxt. and a simple stalked cyst results. In some species there may be a forking of the stalk, but in most cases there is a radial movement from the primary stalked mass, resulting in the formation of heads of small cysts, for example, in *C. crocatus*. In these cysts there may be secondary migrations, a single one toward the center of the cyst in *C. apiculatus* Thaxt., or several, the centers of migration being separated at intervals in a radial line. The last is illustrated by *C. catenulatus* Thaxt., with its heads of cysts in chains.

In the ontogeny of *C. Thaxteri* every stage in the vertical and radial line of evolution relative to this phenomenon of movement

is traversed, and then there is a beginning of a migration of the rods back over the same road before maturity is reached; and this order of development is invariably followed regardless of the conditions of the environment. As the secondary movement in *C. Thaxteri* carries the rods entirely out of the structures that apparently correspond to the cysts of *C. crocatus*, *C. apiculatus*, etc., it seems reasonable to conclude that *C. Thaxteri* stands at the extreme end of the main evolutionary branch of the genus as we know it at present.

Chondromyces Thaxteri, sp. nov.—Pseudoplasmodiis luteo-vel carneo-coloratis; baculis 3–6 $\mu \times 0.5\ \mu$ longis; fructificationibus luteis, 250–750 μ, vulgo circa 350 μ altis; stipitibus plerumque simplicibus, atque interdum ramosis; cystes setaceas, sub-globosas, breviter pedicellatas vel sessiles, 1–7 vel plures numero gerentibus; quae cystes integrae 65–165 μ, vulgo circa 140 μ latae sunt; setae autem sunt conicae, 15–30 μ longae et ad bases 10–12 μ latae.—In fimo cervino nascens in Algonquin Park, Ontario, Canada.

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LITERATURE CITED

1. Baur, E., Myxobakterienstudien. Archiv. Protistenkunde **5**:92–121. 1904.
2. De Kruyff, E., Die Lebensgeschichte von *Myxococcus javanensis*, n. sp. Centralbl. Bakt. **21**:385–386. 1908.
3. Pinoy, M. E., Sur la nécessité d'une association bactérienne pour le développement d'une Myxobactérie, *Chondromyces crocatus*. Compt. Rend. **157**:77–78. 1913.
4. Quehl, A., Untersuchungen über die Myxobakterien. Centralbl. Bakt. **16**:9–34. 1906.
5. Smith, A. L., Myxobacteria. Jour. Botany **1**:69. 1901.
6. ———, Myxobacteriaceae. Trans. Brit. Myc. Soc. **3**:82. 1909.
7. Thaxter, R., On the Myxobacteriaceae, a new order of Schizomycetes. Bot. Gaz. **17**:389–406. 1892.
8. ———, Further observations on the Myxobacteriaceae. Bot. Gaz. **23**: 395–411. *pls. 30, 31*. 1897.
9. ———, Notes on the Myxobacteriaceae. Bot. Gaz. **37**:405–416. 1904.
10. Vahle, K., Vergleichende Untersuchungen über die Myxobakteriaceen und Bakteriaceen, sowie die Rhodobakteriaceen und Spirillaceen. Centralbl. Bakt. **25**:178–260. 1909.

11. ZEDERBAUER, E., Myxobacteriaceae, eine Symbiose zwischen Pilzen und Bakterien. Sitzungsber. Akad. Wiss. Wien 112. 1903.

12. ZUKAL, H., *Myxobotrys variabilis* als Repraesentant einer neuen Myxomyceten-Gattung. Ber. Deutsch. Bot. Gesells. **14**:340. 1896.

EXPLANATION OF PLATES V AND VI

FIGS. 1, 2, 5, 8, 9.—Mature "fructifications" of *Chondromyces Thaxteri;* ×240.

FIG. 3.—An immature stage; ×240.

FIGS. 4, 7.—An immature stage, but later than the one in fig. 3; they represent the cone or finger-like processes formed on the "heads" of the fructification; ×240.

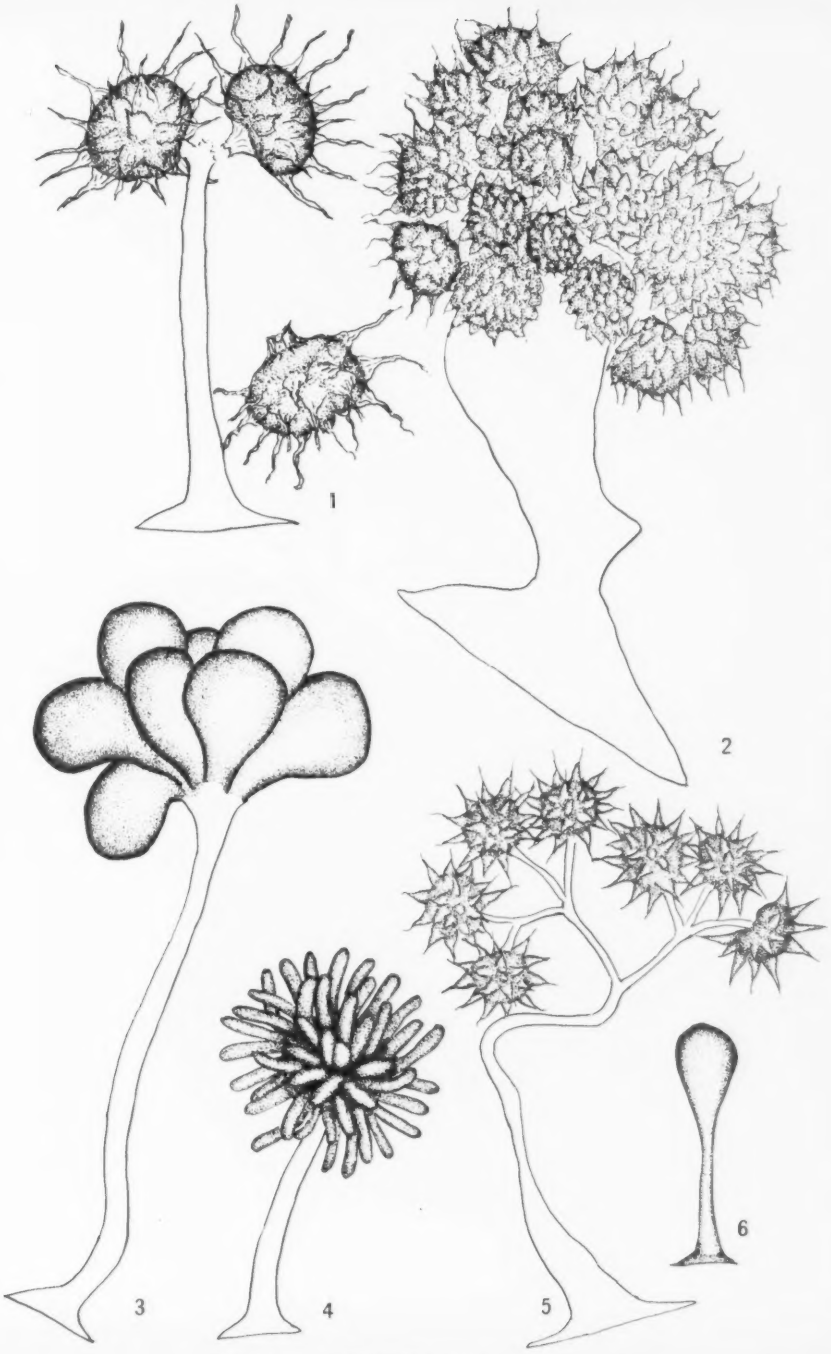
FIG. 6.—An early stage in the heaping up of the rods; ×240.

FIG. 10.—Cysts on the left hand branch are mature, those on the right are rather younger than the ones represented in fig. 3; ×195.

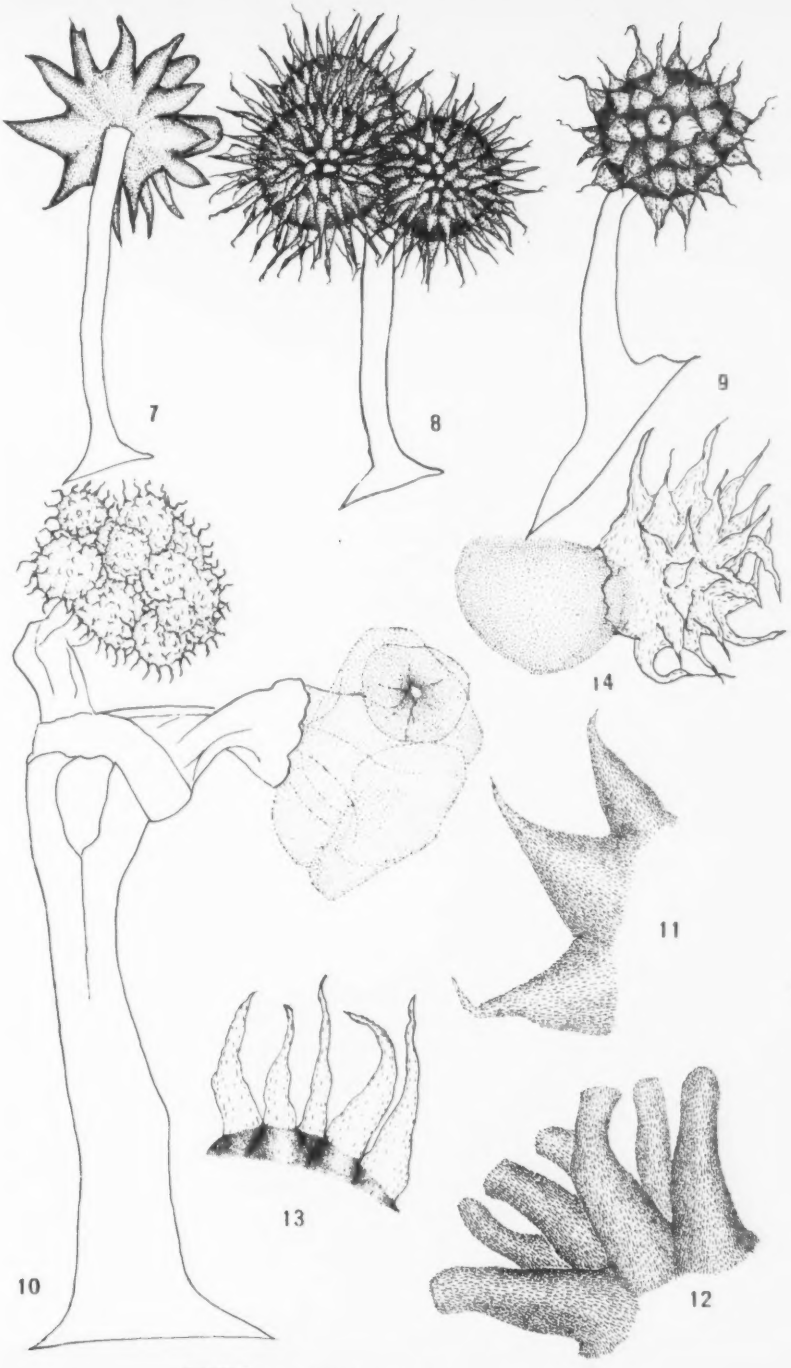
FIGS. 11, 12.—Processes from figs. 7 and 4 respectively, drawn on a larger scale; ×720.

FIG. 13.—Part of a mature cyst (fig. 8) on a larger scale, showing empty husks of processes after rods have migrated back from them; ×720.

FIG. 14.—A germinating cyst; ×720.



FAULL on CHONDROMYCES THAXTERI



FAULL on CHONDROMYCES THAXTERI

THE MODE OF ACTION OF PLANT PEROXIDASES

G. B. REED

(WITH TWO FIGURES)

In a recent paper[1] it was shown that while colloidal platinum markedly accelerates the rate of oxidation of various substances by hydrogen peroxide, it does not ordinarily bring about oxidation in the absence of hydrogen peroxide. Experiments were described, however, which show that when the colloidal metal is charged with oxygen (by making it an anode) it rapidly brings about a certain amount of oxidation. By repeating the charging process at sufficiently frequent intervals colloidal platinum might be made to bring about the oxidation of various substances at a rate approximating that affected by hydrogen peroxide and colloidal platinum. From this it was concluded that the action of the colloidal metal in accelerating oxidation by hydrogen peroxide (that is, its peroxidase action) is due to the taking of oxygen from the peroxide by the metal to form a compound which is a more efficient oxidizing agent than the original peroxide.

This information, gained from a study of a simple peroxidase reaction where the constitution of the catalyzer was known, has made possible an analogous investigation of the more significant and complicated problem of the nature of the peroxidases produced in living tissue. Since, as has frequently been pointed out, the peroxidase action of colloidal platinum closely resembles that of the plant peroxidases, it seemed probable that the mechanism of the reactions must be similar. Accordingly, the experiments which proved fruitful in a study of the platinum reaction have been repeated, as nearly as the material permitted, with certain plant peroxidases.

The very active ferment of horseradish root was first investigated. About 150 gm. of the finely chopped tissue was mixed with twice its volume of distilled water and allowed to stand for 24

[1] REED, G. B., Mechanism of oxidase action. BOT. GAZ. 62:53–64. 1916.

hours. On filtering, a clear, pale yellow extract was obtained, which exhibited very active peroxidase properties.

The peroxidase activity of this extract was determined quantitatively by the method of BACH and CHODAT.[2] To 100 cc. of a solution containing 0.2M potassium iodide and 0.05M acetic acid 20 cc. of the horseradish peroxidase extract was added; the mixture was kept well stirred and at a constant temperature of 15° C., and at frequent intervals portions of 5 cc. were removed and titrated with 0.01M sodium thiosulphate solution (with starch indicator) for the iodine liberated in the oxidation. The values obtained in this way are plotted in the curve *OA*, fig. 1. The rate of oxidation of hydrogen peroxide in the absence of a ferment is shown by the curve *OB* of the same figure. By subtracting the ordinates of the curve *OB* from the ordinates of the curve *OA* we obtain the curve *OC*, which expresses the effect of the catalyzer. For subsequent comparisons the curve *OC* is also plotted as *OC*, fig. 2.

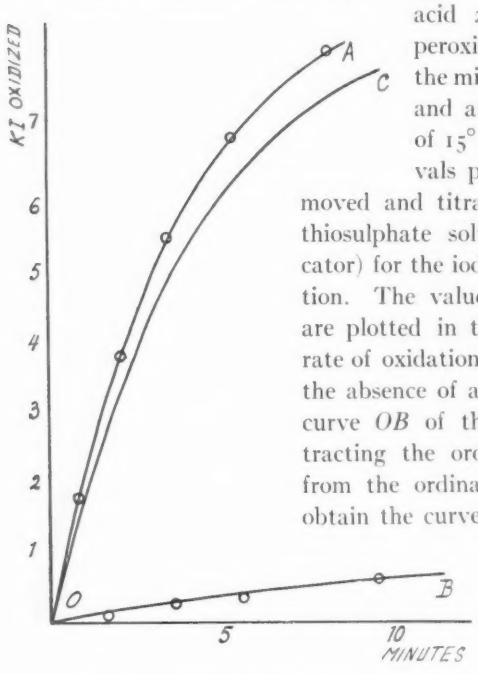


FIG. 1.—Curves of oxidation of potassium iodide by hydrogen peroxide: *OA*, in presence of horseradish peroxidase; *OB*, in absence of a catalyzer; *OC*, the curve resulting when the ordinates of *OB* are subtracted from those of *OA*; ordinates represent number of cc. of 0.01M $Na_2S_2O_3$ required to combine with the iodine in 5 cc. of reaction mixture; abscissae represent time in minutes.

The fresh peroxidase extract in the absence of a peroxide showed no oxidative activity; on adding portions to solutions of potassium iodide and starch or to gum guaiac free from peroxide no oxidation took place. In this respect it was analogous to an ordinary colloidal platinum solution. The platinum may be made

[2] BACH, A., and CHODAT, R., Ber. Deutsch. Chem. Gesells. **35**: 2466–2470, 3943–3946. 1902.

active by charging it with oxygen, either electrically (by making it an anode) or by a chemical method (exposing it to potassium permanganate). Obviously it was not possible to charge the plant peroxidase with oxygen electrically; hence the chemical method was adopted. To about 100 cc. of the same peroxidase solution as that used in the previous experiment concentrated potassium permanganate[3] solution was added drop by drop until the permanganate was no longer reduced, as shown by the color. A small excess of fresh extract was then added to reduce any free potassium permanganate present. The mixture was then filtered free from a small amount of brown precipitate consisting of manganic hydroxide and probably also some organic manganese compounds. In this way a clear solution, somewhat deeper yellow in color than the original extract, was obtained; but this solution possessed a very marked oxidative activity. Solutions of potassium iodide, gum guaiac, or pyrogallol mixed with portions of it, were rapidly oxidized. This new compound was very unstable; when heated rapidly to 60° C. its ability to oxidize gum

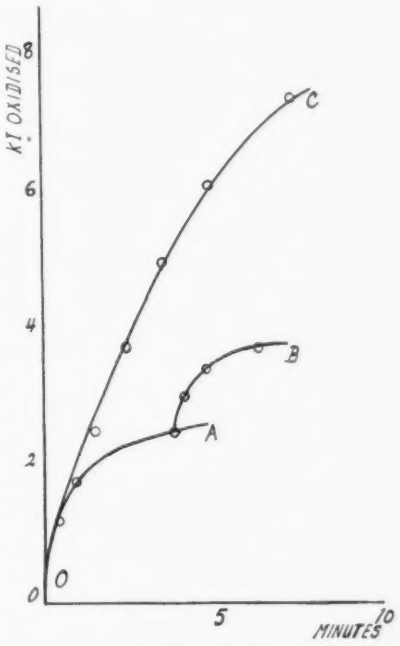


FIG. 2.—Curves showing oxidation of potassium iodide; lower curves *OA* and *AB* represent successive oxidations in the absence of hydrogen peroxide by horseradish peroxidase which had been treated with potassium permanganate at the beginning of each oxidation; curve *OC* represents oxidation by hydrogen peroxide in presence of untreated horseradish peroxidase; ordinates represent number of cc. of 0.01M $Na_2S_2O_3$ required to combine with the iodine in 5 cc. of reaction mixture; abscissae represent time in minutes.

[3] A concentrated solution was used so that the amount added was not sufficient to dilute appreciably the peroxidase extract.

guaiac or potassium iodide directly was destroyed, but its peroxidase activity was still maintained, as shown by a vigorous action on the addition of hydrogen peroxide.

The full significance of the change effected in the extract by treatment with the oxidizing agent was only apparent, however, after determining quantitatively its oxidizing ability and comparing it with the activity of the fresh extract in the presence of a constant supply of oxygen from hydrogen peroxide. This was done in the following manner. To 100 cc. of a solution consisting of equal volumes of 2M potassium iodide and 2M acetic acid 20 cc. of the *treated* horseradish extract was added and the amount of oxidation measured by titrating samples of 5 cc., at frequent intervals, with 0.01M $Na_2S_2O_3$. The curve *OA*, fig. 2, expresses the results in such form that they may be compared with the peroxide reaction which has already been described and which is plotted in the curve *OC* of this figure.

From this comparison it will be apparent that the amount of oxidation by the new preparation is small; but it was found possible after equilibrium had been nearly reached to renew its activity by a second treatment with potassium permanganate. For this purpose the ferment was removed from the potassium iodide solution (after it had been acting 5 minutes) by adding about 2 volumes of 95 per cent alcohol. The small amount of white precipitate which appeared (about half an hour after adding the alcohol) contained the greater part of the peroxidase. This was centrifuged out, washed in 80 per cent alcohol, dissolved in a few cc. of water, and again treated with potassium permanganate in precisely the same manner as before. On adding this preparation to a potassium iodide solution as previously used, oxidation followed at a rate indicated by the curve *AB* (fig. 2). The smaller amount and somewhat slower rate of oxidation are probably due to loss of ferment in the precipitation and subsequent treatment. An attempt to repeat the process a third time was not successful, only a faint cloudiness appearing on the addition of alcohol to the reaction mixture.

By an inspection of the curves (fig. 2) it is apparent that if it were mechanically possible to repeat the process indefinitely the oxidation could probably be indefinitely prolonged. Moreover, by

subjecting the peroxidase to the oxidizing action of the permanganate at sufficiently frequent intervals, it is apparent that the reaction could be made to proceed as rapidly as the curve *OC* (fig. 2), which represents the hydrogen peroxide reaction catalyzed by untreated peroxidase.

Hence we may conclude that just as platinum is recharged with oxygen by hydrogen peroxide, as soon as some of the oxygen has been removed from the platinum by a reducing agent, so the horseradish peroxidase is recharged by hydrogen peroxide under similar conditions.

An investigation of potato peroxidase gave exactly similar results. It may be of interest to note that by treating the peroxidase from the inner tissues of the potato with permanganate it could be made to behave like the oxidase (that is, peroxidase in combination with the oxygenases) from the outer part of the tuber.

It may be concluded, therefore, that in oxidation processes catalyzed by peroxidases two reactions are involved. The peroxidase combines with oxygen from the oxygenases (or from hydrogen peroxide, or possibly from some other source, since it is capable of taking it from potassium permanganate) to form an intermediate compound which is a more energetic oxidizing agent than the original source of the oxygen. The final stage in the oxidation is then affected by this intermediate compound.

A new light is thus thrown on the mechanism of oxidation in living tissues which has so long proved baffling to investigators. The difficulties which beset this field of research are clearly illustrated by the fact that a great amount of careful investigation was necessary before the conceptions of oxygenase and peroxidase could be established. The next step was to elucidate the connection between these entities. This now seems to be accomplished, but it would probably not have been done so soon or so satisfactorily without first making a careful study of the simpler conditions to be found in oxidations which are catalyzed by platinum.

In this connection it is of interest to recall that VAN SLYKE and CULLEN[4] have shown recently that in the fermentation of urea by soy bean urease there is a combination between urea and ferment

[4] VAN SLYKE, D. D., and CULLEN, G. E., Jour. Biol. Chem. **39**:141–180. 1914.

which eventually splits to form carbon dioxide and ammonia. From the course of the reaction these authors have devised a formula which applies to several enzyme reactions which had previously been measured, particularly the hydrolytic action of invertase and lactase. It seems possible that it may prove to be generally true that the catalyzer combines in a definite manner with the reacting substances.

LABORATORY OF PLANT PHYSIOLOGY
HARVARD UNIVERSITY

BRIEFER ARTICLES

THE HALL HERBARIUM

Students of botanical taxonomy in the Central States and America are to be congratulated upon the donation to the Field Museum of Natural History of the herbarium of the late ELIHU HALL. The family of Mr. HALL, after long deliberation, decided that in the herbarium of the Field Museum the botanical work of their father would be advantageously preserved in a highly referable form; his individuality maintained; and his material most carefully organized.

The herbarium of Mr. HALL comprises about 35,000 sheets, particularly rich in western and southwestern United States plants. It contains the original HALL plants of Texas, the American Plains, and Oregon; the HALL and HARBOUR collections of the Rocky Mountains and American Plains; and a unique series of western species grown at Athens, Illinois, from seeds of plants collected from Texas northward to Oregon. The latter series is carefully labeled, so that in every case the original plant may be directly compared with the specimen of the same as grown in the new environment.

Mr. HALL was an active and careful conductor of exchanges from 1858 to 1870, a fact that results in large series of plant specimens contributed to his herbarium by BOLANDER, California; BRANDEGEE, Colorado; BUCKLEY, Texas; CANBY, various localities; CHAPMAN, Florida; CLINTON, New York; COUTHOUY, Ecuador; CURTISS, Virginia and Florida; FAXON, Florida; FENDLER, New Mexico; FORSHEY, Texas; GARBER, Florida; GATTINGER, Tennessee; HALE, Louisiana; HOWELL, Oregon and Washington; JAMES, California; JONES, Utah; LOOMIS and CROOM, North Carolina; MACOUN, Canada and British Columbia; MOHR, Alabama; PARRY and PALMER, Mexico; RAVENEL, South Carolina; RIDDELL, Texas; SHORT, Kentucky; TORREY, various localities; VASEY, Illinois and California; VOLLUM, Texas; WOLF, Colorado; WRIGHT, New Mexico; MCOWEN, South Africa; MUELLER, Australia; and various other American and foreign collectors and herbarists.—C. F. MILLSPAUGH, *Field Museum of Natural History, Chicago.*

CURRENT LITERATURE

BOOK REVIEWS

A textbook of physiological chemistry

On account of the rapidly growing importance of physiological chemistry in modern plant physiology, a recent textbook by MATHEWS[1] will be found of particular interest to plant physiologists. Much of this large volume is of equal interest to plant and animal physiologists. Those features which concern the plant physiologist more especially are as follows.

The point of view seems more that of the biochemist than previous texts on this subject, which have been treated almost exclusively from the standpoint of the animal physiologist. This is doubtless due to the more extensive treatment of those phases of the subject not exclusively of interest to the animal physiologist, namely, the chemistry of the carbohydrates, fats, and proteins, and the physical chemistry of the cell. These subjects constitute part I (pp. 265). Some plant physiologists, perhaps selfishly, had hoped for even more extensive treatment of the more general phases of the subject.

Part II deals with "the mammalian body considered as a machine," and while it is of more direct interest to the animal physiologist, there are chapters which interest the plant physiologist as well, namely, the chapters entitled "Animal heat" and "Metabolism under various conditions." In the former chapter there is given a summary of our present knowledge of respiration and a historical résumé of its development. The brief historical discussion which the author has given in connection with the treatment of various phases of the whole subject of physiological chemistry seems especially commendable. The statement made early in the text that the energy of the body comes "immediately from the union of living matter or its constituents with oxygen" indicates that he leans toward the PFLÜGER-VERWORN conception of respiration. This conception and the opposing view of HOFMEISTER are treated in the latter chapter under respiration.

Part III is devoted to practical work and methods. An admirable laboratory course dealing with the general phases of the subject and a course of special methods in quantitative analysis of plant and animal tissue form the basis. In the treatment of this practical work the author gratefully acknowledges the able assistance of his colleague F. C. KOCH, under whose direction, for the most part, these courses have been developed. A separate index to part III is an excellent feature.—LEE I. KNIGHT.

[1] MATHEWS, ALBERT P., Physiological chemistry, a textbook and manual for students. 8vo. pp. 1040. New York: Wood. 1915.

Artificial parthenogenesis and fertilization

This belated notice of LOEB's book[2] was suggested by the fact that it does not seem to have become known in botanical circles to an extent worthy of its significance. The material is almost wholly zoological, but the book deals with such fundamental problems that it cannot fail to be of interest to all biologists. Chap. iv, on "Hydrolytic processes in the germination of oil-containing seeds," and chap. xxix, on "Artificial parthenogenesis in plants," are of special botanical interest. It must not be inferred from the title that LOEB attempts a systematic presentation of the phenomena in animals and plants; on the contrary, he presents for the most part his own results, his own methods of work, and his own points of view; and as these have concerned so preponderatingly the subject of artificial parthenogenesis, fertilization comes in for relatively scant attention. Nor must it be supposed that the book represents any definitive standpoint with reference to the problems presented. LOEB has already discarded some of the working hypotheses outlined in his book, and has gone beyond the standpoint of the book in other particulars.

The book is of interest in two particular respects: (1) as a presentation of the author's own classical work in this field which he has done so much to illuminate, and (2) as a splendid example of biological analysis which takes a particular genetic process out of the realm of morphology and attempts a thorough physiological analysis, relating it as far as possible to physico-chemical processes on the one hand and to seemingly remote biological processes on the other hand. The author is so fresh and original in his point of view that one cannot fail to profit by the presentation as a study in scientific method. There are few biologic books in which this connection between hypothesis and discovery is more convincingly illustrated.—F. R. LILLIE.

Phoradendron

Probably no genus of plants has been given more elaborate and detailed treatment than the exclusively American genus *Phoradendron* has received in the recent monograph by TRELEASE.[3] It is the record of a prolonged and painstaking study of a difficult and poorly known group. In this monograph 277 species are recognized, and their geographical distribution is very interesting. The two primary groups are named "Boreales" and "Aequatoriales," and the names indicate their distribution. Of the 66 species of the former, 28 occur in the United States, 48 in Mexico, 2 in Central America, and none in the West Indies and South America; while of the 211 species of the latter, none occur in the United States, 29 in Mexico, 20 in Central America, 38 in the West Indies, and 134 in South America. The new species are remarkably

[2] LOEB, J., Artificial parthenogenesis and fertilization. The University of Chicago Press. 8vo. pp. x+312. *figs.* 87. 1913.

[3] TRELEASE, WILLIAM, The genus *Phoradendron*. Large 8vo. pp. 224. *pls.* 245. Urbana: University of Illinois. 1916. $2.50.

numerous, reaching 102, of which 29 are Boreales and 73 Aequatoriales. This well emphasizes the fact that the genus was very poorly understood.

The book includes all the features of a complete monograph in the way of discussion, keys, descriptions, citations of stations and exsiccatae, etc. The discussion includes such subjects as a historical account of the taxonomy of the group, an extended analysis of the characters used, the facts of parasitism, the origin of the genus, and the range of the species. The numerous plates, which are unusually clear photographic reproductions of types and authentic specimens, approach in value for reference a set of actual specimens.—J. M. C.

Soils

RUSSELL[4] has written another most interesting little book upon soils. His position as director of the famous Rothamsted Experiment Station, which has probably contributed more to our knowledge of soils than any other institution, and his established ability as a writer and as an authority on soil subjects, lead one to expect much from a book written by him, and in the present instance this expectation is fully justified. While the book is directly practical, dealing with matters that tillers of soils need most to know, it leaves the reader with a clear grasp of the main principles established by science and practice in this field. RUSSELL shows a happy breadth of view in treating the subject in this as in his earlier works. Some of our American writers on soils can profit by his example in this respect.

The revision of RUSSELL's monograph on *Soil conditions and plant growth*[5] should have been noted at an earlier date. The first edition has already been reviewed in this journal.[6] While the second edition involved no essential changes, the third edition has a new chapter on the relationship between the micro-organic population of the soil and the growth of plants, also added sections in various other chapters of the book, along with other modifications that bring the book up to date. The book stands alone as a clear, terse, exact statement of the soil conditions in relation to plant growth.—WILLIAM CROCKER.

MINOR NOTICES

Microscopy of vegetable foods.—A second edition of WINTON's volume under this title has appeared.[7] The general scope and purpose of this valuable

[4] RUSSELL, E. J., A student's book on soils and manures. pp. ix+206. *figs.* 3. Cambridge Press. 1915.

[5] ———, Soil conditions and plant growth. VIII. pp. viii+190. *figs.* 9. 3d ed. Longmans, Green, & Co. 1915.

[6] BOT. GAZ. **55**:167–168. 1913.

[7] WINTON, ANDREW L., The microscopy of vegetable foods, with special reference to the detection of adulteration and the diagnosis of mixtures. With the collaboration of Dr. JOSEF MOELLER and KATE BARBER WINTON. 2d ed. Imp. 8vo. pp. xiv+701. *figs.* 635. New York: John Wiley & Sons. 1916. $6.50.

work were stated in this journal[8] upon the appearance of the first edition. The second edition has incorporated the results of ten years of activity in the examination of human and cattle foods. Among the features of the edition are additions to the sections on wheat and flour; a complete revision of such parts of the chapter on oil seeds as treat of mustards, rapes, cruciferous weed seeds, and linseed; a description of the histology of alfalfa; and a revision of the sections on pomes and drupes. The arrangement of material, analytic keys, lists of adulterants, and the suggestions as to diagnosis are of great practical service in the campaign against sophistication by unscrupulous manufacturers and dealers.—J. M. C.

Plant anatomy.—STEVENS[9] has published a third edition of his well known *Plant Anatomy*, which speaks well for the growing interest in the subject, and also for the quality of the book. The other editions were reviewed in this journal,[10] so that the scope of the work has been described. In the present edition the most important addition is a section on the phylogeny of the vascular bundle, which could not be omitted from the plant anatomy of today. Naturally, there are also additions that include various improvements in technique. The author is a thoroughly good teacher, which means a well organized book and clear presentation.—J. M. C.

The first American Botany.—SAMUEL N. RHOADS has published a facsimile reprint of YOUNG'S *Catalogue d'arbres arbustes et plantes herbacées d'Amérique*, published in Paris in 1783, which is claimed to be the earliest published book written by an American botanist and devoted exclusively to American plants. The editor has given a prefatory account of the author, WILLIAM YOUNG, Jr., of Philadelphia, adding a very interesting personality to the known list of pioneer American botanists, and one whose chronological position in our American literature should be recognized. The book is privately printed in Philadelphia.—J. M. C.

North American flora.—The fifth part of Vol. 9 continues the presentation of the Agaricaceae by MURRILL,[11] 10 genera being presented, which include 311 species, 134 of which are described as new. The largest genus is *Prunulus*, with 106 species, 53 of which are new; the next is *Gymnopus*, with 93 species, 45 of which are new. New species are also described in *Geopetalum* (5), *Micromphale* (2), *Omphalopsis* (14), and *Omphalina* (15).—J. M. C.

[8] BOT. GAZ. **41**:300. 1906.

[9] STEVENS, W. C., Plant anatomy, from the standpoint of the development and functions of the tissues and handbook of microtechnic. 3d ed. 8vo. pp. xviii+399. *figs. 155*. Philadelphia: Blakiston's. 1916. $2.50.

[10] BOT. GAZ. **46**:306. 1908; **50**:470. 1910.

[11] MURRILL, W. A., North American Flora **9**: part 5. pp. 297–374. Agaricales: Agaricaceae (pars), Agariceae (pars). New York Botanical Garden. 1916.

NOTES FOR STUDENTS

Periodicity in tropical trees.—KLEBS[12] has added some important evidence on the much disputed question of the cause of periodicity in tropical trees. It has been observed that some tropical trees in the very nearly constant climate of their native habitat show no vegetative periodicity, while most of them show an alteration of growth and rest in some form or other. VOLKENS and others attribute this behavior or difference in behavior to hereditary characters; while KLEBS thinks of it as the result of external (nutrient) conditions acting upon the specific structure (hereditary characters) of the plants. KLEBS would say that the course of development of any plant or plant primordium is determined by two things, specific structure of the unit and the condition under which it develops. Every such unit has many potentialities so far as course of development is concerned; the one realized depends upon the condition under which it develops. The reviewer feels that the dual nature of the determiners of the course of development of the individual should sink deeply into the minds of all botanists. It will clear away much foggy thinking frequently manifested by such statements as "this character is due to heredity" and "that one to environment." *Every character is due to both.* Here the speculative philosopher's attempt to get at the *one essential factor* fails, for there are two.

The work was done in the greenhouse at Heidelberg on the following forms in the main: *Terminalia Catappa*, *Theobroma Cacao*, *Albizzia stipulata*, and *Pithecolobium Saman*. KLEBS states that since growth depends upon a great number of factors, rest can be procured by any one of these sinking to a certain minimum, and this minimum varies greatly for the different species. KLEBS worked especially with light and nutrient salts. The light intensity and its daily durations at Heidelberg gradually fall to a low minimum late in December. With good salt supply there were 3 types of response to this seasonal light minimum: (1) buds were formed and unfolded continuously throughout the year, but the leaves were smaller and their daily growth less during the time of minimum light (*Terminalia*, *Albizzia*, *Pithecolobium*); (2) leaves were formed throughout the season, but these did not unfold normally from November to January (*Theobroma*); (3) plants rested during the months of minimum light (*Eriodendron anfractuosum* and probably *Sterculia*).

The growth inhibiting or rest producing effect of diminished light may be shown in summer. This is most marked in complete darkness, which readily throws even *Albizzia* and *Pithecolobium* into rest. *Albizzia* continues to vegetate in darkness if it has much stored food, so that the effect of diminishing light seems to be related to reduced food supply and not to such specific action as KLEBS found for the beech. Limited light and limited salt supply may act together in producing rest. *Albizzia*, *Pithecolobium*, and *Terminalia* were

[12] KLEBS, G., Über Wachstum und Ruhe tropischer Baumarten. Jahrb. Wiss. Bot. **56**:734–792. 1915.

thrown into rest periods at the winter period of minimum light if they were pot bound, but not with good salt supply. The part played by specific structure was evident here, for light amounts that produced continuous unfolding in buds of *Terminalia* gave buds that did not unfold in *Theobroma*, and complete rest in *Eriodendron*.

Intense photosynthesis resulting in high carbohydrate supply produces rest if salts are insufficient. One growth period may exhaust the salt supply, thus producing rest which lasts until the salt supply rises because of non-consumption and induces a second growth period. KLEBS suggests that this is the condition that throws into dormancy the buds formed in the spring on trees growing in our climate. The salts have been reduced by the formation of stems and leaves and the carbohydrates are abundant, owing to the activity of the newly formed leaves. He suggests that high photosynthesis along with low salt supply may produce dormancy in a similar way in trees of the tropics Several workers have shown that older individuals of a tropical species are more likely to show a rest period than young ones. This KLEBS attributes not to age so much as to limited nutrients conditioned by a multiplication of buds dependent upon a single absorptive and conductive system.

KLEBS criticizes JOST and SIMON for classifying salts with anesthetics as mere stimuli to growth, rather than thinking of their nutrient significance. He speaks of salt addition as a quantitative increase of an already present and *absolutely necessary growth factor*. In this connection he emphasizes the error of assuming that the soil is a constant factor. In such a complex system of organisms and organic and inorganic materials in ever shifting equilibria one must expect periodic changes in supply of nutrient salts as well as in other factors. The frequently observed fact that different individuals of the same species in the tropics show marked differences in vegetative periodicity may be due to the soil factor.

KLEBS speaks of throwing *Pithecolobium* into rest at will (by salt reduction) or active growth (by salt addition) with the same sort of ease as *Vaucheria* can be caused to reproduce by zoospores on the one hand, or by oospores on the other; or water to assume the form of a solid under one condition and that of a liquid under another condition. His evidence leaves little doubt that the tropical trees in general can be made to behave quite like *Pithecolobium*. It seems that KLEBS has established his general contention of the dual determination of periodicity in these forms, but there are some minor conceptions that are less happy.

He classifies all nutrient salts together as if they all have the same effect upon the course of development, while agriculturists have fully demonstrated that nitrates and phosphates in some respects have opposite effects. He implies that salts have their effects mainly as nutrients (building materials), while the extensive work on antagonism probably deals with general physical or colloidal effects, and there is evidence that metallic ions are of importance in catalysis. Moreover, it is not yet shown whether high nitrate supply induces

vegetation and succulence through materials (proteins, etc.) built from it or through its lyotropic effects, and whether the partially contrasting effects of phosphates depend upon the first or second condition. Periodicity in salt absorption which has been observed in trees and grains is also minimized It seems evident that to get far back of the general proposition which KLEBS has apparently proved, there is need of a careful study of internal conditions of the plant, anatomical, chemical, and microchemical, as well as the application (by injection or otherwise) of various salts and carbohydrates and products manufactured from them to be sure of the effective agents.—WILLIAM CROCKER.

Morphology of Gnetum.—THOMPSON[13] has done a much needed service in making a thoroughgoing investigation of *Gnetum*. We have had too meager information in reference to this important and puzzling genus, owing mainly to the difficulty of obtaining material representing any extended and close series of stages. THOMPSON obtained his material during a visit to the Malay Archipelago, and chiefly from the Botanic Garden at Buitenzorg and the adjacent country. As a consequence, he has published an extended account of all the structures related to reproduction. Several species were investigated, and especially two species that represent the extremes of variation. The details are so numerous that they cannot be repeated in a review, but some of the more important facts may be mentioned. Certain strobili were found in which the flowers are arranged in a spiral, and are thus very suggestive of catkins of Amentiferae. In the development of the megasporangium the 3 envelopes arise in acropetal succession, and the micropylar tube, which THOMPSON regards as a style, develops conducting tissue. No vegetative cells appear in the development of the male gametophyte, and the microspores frequently germinate in the micropylar tube at a distance from the nucellus. At fertilization stage the female gametophyte consists of approximately 256 or 512 free nuclei; and after the entrance of the pollen tube rapid divisions occur, multinucleate compartments being formed and all the nuclei in each compartment fusing.

THOMPSON concludes that the strobili of *Gnetum* are closely related to the catkins of the Amentifereae; that the flowers are reduced from a bisporangiate condition; that the inner "envelope" of the ovulate flower is an ovary homologous with that of the angiosperms and bearing a true style (the micropylar tube); that the female gametophyte is gymnospermous in its early stages and angiospermous in the later ones; that the fusion of nuclei preceding endosperm formation is a forerunner of that in angiosperms; that the angiosperms have been derived from ancestors very much like modern Gnetales, and that, in fact, the genus *Gnetum* should probably be classified with angiosperms.—J. M. C.

[13] THOMPSON, WALTER P., The morphology and affinities of *Gnetum*. Amer. Jour. Bot. 3:135–184. *pls. 2–7*. 1916.

Orthogenetic saltation.—BENEDICT[14] has undertaken a study of the variations of the Boston fern (*Nephrolepis exaltata bostoniensis*). His title is selected to emphasize two points: (1) the variations are discontinuous (saltation), and (2) they occur in a definite series along a few limited lines (orthogenetic). It seems that from this fern there have arisen more than 100 forms in less than 15 years. The present paper is concerned chiefly with a record of the facts. Uniform and highly developed cultural conditions are thought to favor the preservation of variations which under wild conditions would be eliminated.

The variations are classified as progressive and regressive, implying in the one case increasing departure from the parent form and in the other case a return toward the parent form. Progressive variations have appeared along three main lines: (1) increasing division of the leaf, observed through 5 vegetative generations; (2) increasing ruffling of the pinnae, observed through 3 generations; and (3) dwarfing, observed through 3 generations. Regressive variations rarely if ever show a complete return to the parent form. The coefficient of variation for progressive variations is very low, probably between 1:1,000,000 and 1:1,000; while regressive variation is much more common. The variations are all discontinuous and the differences are said to be as great as those existing between many wild species of ferns. The main difference between these variations and those shown by wild forms is that the former do not survive natural conditions, the variation usually being accompanied by diminished vigor.—J. M. C.

Anomalous endosperm and the problem of bud sports.—EMERSON[15] has recorded some new and interesting cases of hybrid maize kernels in which half of the endosperm shows a different combination of Mendelian characters from the other half, although it is obvious, from the fact that both parts show xenia, that the endosperm as a whole is due to double fertilization. He discusses two hypotheses, either of which might account for such kernels, namely, EAST and HAYES' hypothesis of somatic segregation and his own hypothesis of somatic mutation. A third, suggested years ago by COULTER and CHAMBERLAIN, seems to the reviewer more plausible than either. In certain plants it has been observed that the division of the primary endosperm nucleus begins before the constituent nuclei have lost their identity. If such a condition were to be demonstrated in maize, we would have a mechanism for the production of mosaic endosperm which could be called neither segregation nor mutation, in the sense in which EMERSON obviously uses the latter term

[14] BENEDICT, R. C., The origin of new varieties of *Nephrolepis* by orthogenetic saltation. I. Progressive variations. Bull. Torr. Bot. Club **43**:207–234. *pls. 10–15.* 1916.

[15] EMERSON, R. A., Anomalous endosperm development in maize and the problem of bud sports. Zeitschr. Ind. Abstamm. u. Vererb. **14**:241–259. 1915.

when he speaks of the origin of a recessive Mendelian variety as a bud sport. The hypothesis of incomplete triple fusion is in a way a compromise between the inapplicable and discarded older hypotheses involving entire suppression of the triple fusion and the later ones involving no gross cytological aberrations whatever. Although heartily in accord with EMERSON'S well-considered views in regard to somatic mutation in general, the reviewer must confess to a distrust of drawing a close parallel between bud sports and anomalous endosperm development.—H. H. BARTLETT.

Tubers of Nephrolepis.—SAHNI[16] has investigated the vascular anatomy of the tubers borne on the underground stolons of *Nephrolepis*, and has uncovered a very interesting situation. The vascular strand of the stolon penetrates the base of the tuber as a protostele for a short distance, and then expands into a funnel, acquiring in succession internal phloem, pericycle, endodermis, and "ground tissue." Later the funnel-like stele breaks up, at the same time expanding enormously, into a hollow network of ribbon-like strands (each concentric in structure) inclosing gaps of irregular shape and size. These strands converge again into a single protostelic strand, which usually ends in the apical "mamelon." Root strands arise promiscuously from this reticulate stele. SAHNI calls attention to the fact that the conspicuous gaps that appear in this latticed cylinder cannot be explained as leaf gaps, since there is no trace of leaves on the tuber. He suggests that it is a case of a solid stele dilated sufficiently to transform it into a hollow network. TANSLEY has suggested that it is the dilation of a protostele that converts it into a siphonostele.—J. M. C.

Soil Science.—Many will welcome the founding of the new journal *Soil Science*. It is published (first number January 1916) at Rutgers College, with JACOB G. LIPMAN as editor-in-chief, and NICHOLAS KOPELOFF and CARL R. WOODWARD as assistant editors, along with 23 consulting editors, representing experts in this line from various parts of the United States and from 9 foreign countries. The editor-in-chief outlines the scope of the journal in the following statement: "*Soil Science* is to be devoted to problems in soil physics, soil chemistry, and soil biology. Papers dealing with problems in plant physiology, agronomy, bacteriology, or geology will be accepted only when they may contribute directly to our knowledge of soil fertility." He feels that greater cooperation will be gained among American workers on soil problems by a common channel of publication, in contrast with the previous distribution of articles through a number of American and several foreign journals. Promptness of publication is also of great importance. No doubt this will bring a welcome if only a slight relief to overcrowded journals in a number of lines.—WILLIAM CROCKER.

[16] SAHNI, BIRBAL, The vascular anatomy of the tubers of *Nephrolepis*. New Phytol. 15:72–80. *figs.* 3. 1916.